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COMPARATIVE REACTION OF OAT VARIETIES IN THE SEEDLING AND MATURING STAGES TO PHYSIOLOGIC RACES OF PUCCINIA GRAMINIS AVENAE, AND THE DISTRIBUTION OF THESE RACES IN THE UNITED STATES [1]

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INTRODUCTION

In the course of studies carried out at the Minnesota Agricultural Experiment Station on the reaction of different varieties and hybrids of oats to the stem rust fungus, *Puccinia graminis avenae* Eriks. and Henn., the following questions arose: (1) Would the results obtained from inoculating seedling plants with specific physiologic races correspond with the results procured when plants of the same varieties approaching maturity were inoculated with identical races of the fungus? (2) With how many and with which particular physiologic races is the oat breeder likely to be confronted in the United States in any given area or in the country as a whole? Field practice might be considerably simplified and expedited if heterozygous and homozygous F_3 lines could be classified on the basis of seedling reaction in greenhouse tests; and the problem of developing resistant varieties might be facilitated if adequate knowledge were available regarding the prevalence and distribution of the different physiologic races of oat stem rust.

Results obtained by various workers in the past have, in the main, indicated essential agreement between seedling and adult-plant reaction to rust within the limits of a given crop variety, although a few notable exceptions have been reported. It was to investigate this point for each known physiologic race of *Puccinia graminis avenae*, tested on standard varieties and promising strains of oats, that the present study was undertaken. The work was greatly simplified by the use of purified cultures of all known physiologic races of oat stem rust, by the availability of some very important varieties and strains of oats commercially grown or in the process of development, and by the accumulation of rust-survey data over a period of many years.

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REVIEW OF LITERATURE

The question of the relation between the reactions of seedling and adult plants to rust has long attracted the attention of plant pathologists and plant breeders. While in most cases there is evidence of a close correspondence, in some instances the interaction between host and parasite apparently undergoes modification as the plants approach maturity. Thus, certain varieties of wheat that were highly susceptible to specific physiologic races of *Puccinia graminis tritici* Eriks. and Henn. in the seedling stage withstood stem rust epidemics under field conditions exceedingly well, even though some of these races were present in considerable abundance. Thus far, no such discrepancies have been reported in the great majority of cases where oat varieties were tested.

HISTORY OF COMPARATIVE REACTION IN WHEAT

As early as 1914, Stakman (*30*)[3] reported on the relatively high susceptibility of Einkorn and Iumillo (durum) wheat, in the seedling stage, to stem rust under greenhouse conditions, and their extreme resistance as adult plants in the field. Later, Stakman and Piemeisel (*33*) noted that certain grasses, such as some species of *Agropyron* and *Elymus*, were completely susceptible when young but much less so when older. While Melchers and Parker (*22*) found that, in general, seedlings inoculated in the greenhouse reacted to the rust organism in a manner similar to that of adult plants in the field nursery, they were aware that plants showing certain effects when inoculated in the seedling stage in the greenhouse may respond very differently when subjected to the same rust organism in the heading stage under field conditions.

Hayes and Aamodt (*16*) pointed out that certain wheat strains were at least moderately resistant to stem rust in the field, although under greenhouse conditions these strains, as seedlings, were susceptible to a number of races of *Puccinia graminis tritici*. Hursh (*18*) called attention to the difference between the stem rust reaction of Acme seedlings in the greenhouse and that of plants of the same variety on approaching maturity in the field. Harrington (*13*) concluded from the reaction of some durum crosses that there was "a complete lack of correlation between either resistance or susceptibility in the field and the reaction of the hybrids to form 21 in the greenhouse." Hayes, Stakman, and Aamodt (*17*) demonstrated rather conclusively that certain varieties and hybrids of wheat, although susceptible in the seedling stage in the greenhouse, possessed appreciable resistance in the field, even where the stem rust epidemic was induced by many physiologic races. Aamodt (*1, p. 217*) states:

> A greenhouse test is necessary to determine the mode of inheritance of the reaction of a variety or hybrid to particular physiologic forms. A field test is necessary in order more fully and accurately to determine varietal reaction to rust under the particular environmental conditions where the variety is to be grown.

Levine (*19*) reported that during the 5-year period 1919–23 the reaction of wheat varieties in a given field nursery was generally the same as in the greenhouse to pure cultures of the physiologic races isolated from such a nursery. "This, however, was not always true

[3] Reference is made by number (italic) to Literature Cited, p. 727.

of Acme, Monad, Pentad, Kota, and Vernal, which often escaped infection under field conditions" (*19, p. 117*). Goulden, Neatby, and Welsh (*12*) showed that resistance in the maturing stage was inherited independently of seedling resistance and, consequently, concluded that the two types of resistance were quite distinct.

Harrington and Smith (*14*) observed that while "the use of seedling results as indications of after-heading reactions would have to depend upon the variety under consideration", there generally was a positive correlation between the seedling reaction and the degree of infection after heading. Popp (*28*) reported that Acme and H–44 developed resistance to race 21 as they approached maturity, although in the seedling stage they were susceptible to the rust. Newton, Johnson, and Brown (*25*) confirmed the fact that in the case of Acme the seedling reaction cannot be considered a criterion of its rust reaction at maturity. Hart (*15*) demonstrated that some varieties of wheat (notably Hope, Webster, Acme, and Velvet Don) may in part owe to the behavior of their stomata their resistance to stem rust in the field. Goulden (*10*) pointed out that the inheritance of maturative reaction is entirely independent of seedling reaction, insofar as H–44 and Pentad are concerned, and that this inheritance is of a simple nature. Results obtained by Goulden and Neatby (*11*) indicate that the maturative resistance of H–44–24 and Hope is effective, or nearly so, with respect to all known physiologic races. Neatby (*24*), after a study of the comparative reactions of seedling and adult plants of three crosses (Marquillo×Reward, Garnet×Marquillo, and Garnet× Double Cross), came to the conclusion that—

> the inheritance of the field reaction to stem rust, as determined by percentage infection, in these crosses is mainly if not entirely controlled by the factors which govern in the inheritance of the seedling reaction to form 21 in the greenhouse, as determined by pustule type.

In Uruguay, according to Gassner (*7*), field infection of leaf rust is most severe on young wheat plants, whereas stem rust develops more profusely as the plants grow older. The behavior of crown rust there is similar to but less marked than that of oat stem rust. Thus, in Uruguay, within the same variety of host plant the degree of infection with a given rust varies to some extent according to the age of the plant.

HISTORY OF COMPARATIVE REACTION IN OATS

There are not, to the authors' knowledge, any studies reported in the literature designed primarily to show the relation of seedling and adult-plant reactions in varieties of oats to specific races of stem rust. A few investigators, however, have considered this problem incidentally with regard to both stem rust and crown rust. Thus, Parker (*26*) reported on the reaction of more than 120 lines of oats to both rusts. The effects of each rust on oat seedlings and maturing plants were studied. He states (*26, p. 13*):

> In 80 out of the 122 cases the results at two distinct periods in the life of the host plant have led to identical conclusions as to the susceptibility of the variety. In some of the resistant varieties, also, both seedlings and mature plants gave the same evidence of resistance, though the results are not always in agreement.

In subsequent experiments on rust reaction in oats made by the same author (*27*), the variety Sixty-Day proved to be about equally susceptible in the adult stage as in the seedling stage when inoculated

with stem rust. Similar results were obtained with crown rust. Durrell and Parker (*6*) found that oats in the seedling and heading stages reacted similarly to crown and stem rust in greenhouse and field tests. Stakman, Levine, and Bailey (*31*) stated that "as a result of previous work, it is safe to conclude that the reaction of seedlings to rust forms is a fairly accurate index of the reaction of older plants" where oat stem rust is concerned. It appears from their description of methods, that Mackie and Allen (*21*) might have noted a positive correlation between seedling and adult plant reaction, though no definite statement concerning this point was made by them.

The results obtained by Bailey (*2*) on the reactions of seedlings and adult plants of oats to stem rust indicate the existence of a close agreement in the material he used. Gordon (*8*) found that some 230 varieties and strains of oats of American and European origin were all highly susceptible in the seedling stage to race 6 of *Puccinia graminis avenae*, and that the seedlings of all but 1 of these varieties were about equally susceptible to race 4. In a field test these two rust races were similarly virulent on adult plants. Levine, Stakman, and Stanton (*20*) noted a general agreement in the reaction of certain varieties of oats, grown in uniform rust observation nurseries at various experiment stations during the 5-year period 1923–27, with the reaction of seedling plants grown in the greenhouse to physiologic races of stem rust isolated from these nurseries. Welsh (*40*) demonstrated that in all cases where resistant lines of oats were tested with races 1, 2, 3, 5, and 7 of *P. graminis avenae*, in the greenhouse and in the field, there was full agreement in the reaction of the seedlings with that of the maturing plants. According to Gordon (*9*), essentially the same results were obtained with seedling and adult plants of Joanette strain, when inoculated with identical physiologic races of stem rust of oats and cultured under comparable temperature conditions. Smith (*29*) noted essential agreement between seedling reaction to stem rust and crown rust of oats, grown under greenhouse conditions, and the reaction of adult plants of the same F_3 families when grown in the field, in the environment existing at University Farm, St. Paul, Minn.

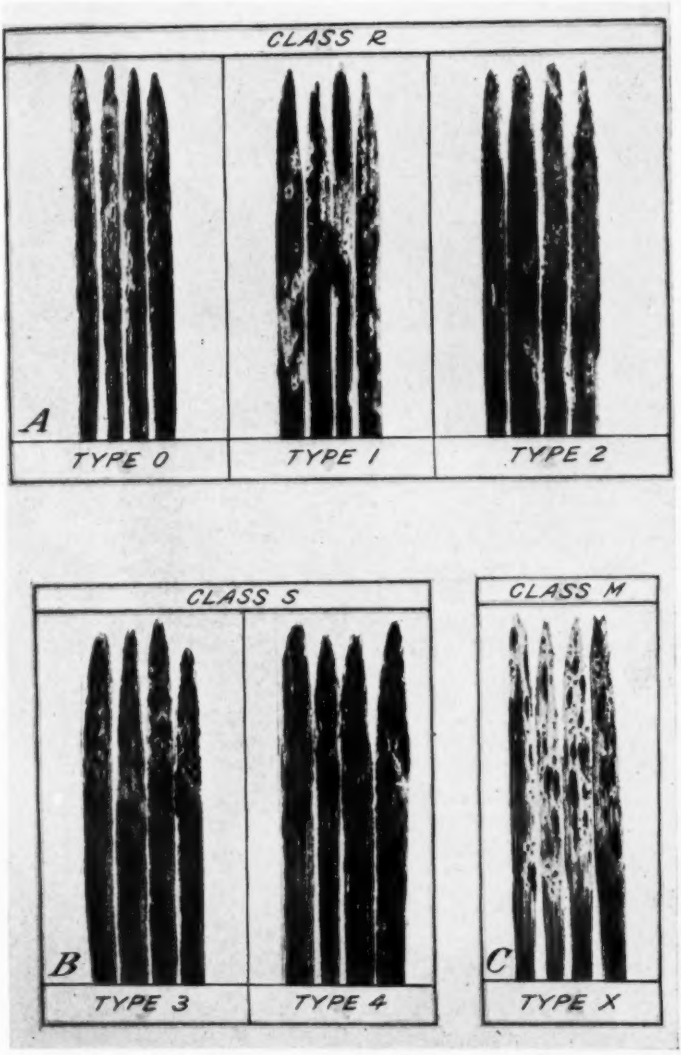
The foregoing review reveals a striking uniformity in the results obtained by various workers regarding the relative susceptibility to rust of oat plants at different stages in their development. In virtually all cases there appeared to be a harmonious agreement in the reaction of seedling and adult plants of oats to both stem and crown rust. No such correlation prevailed in every instance reported for wheat varieties, as was brought out in the preceding section.

MATERIALS AND METHODS

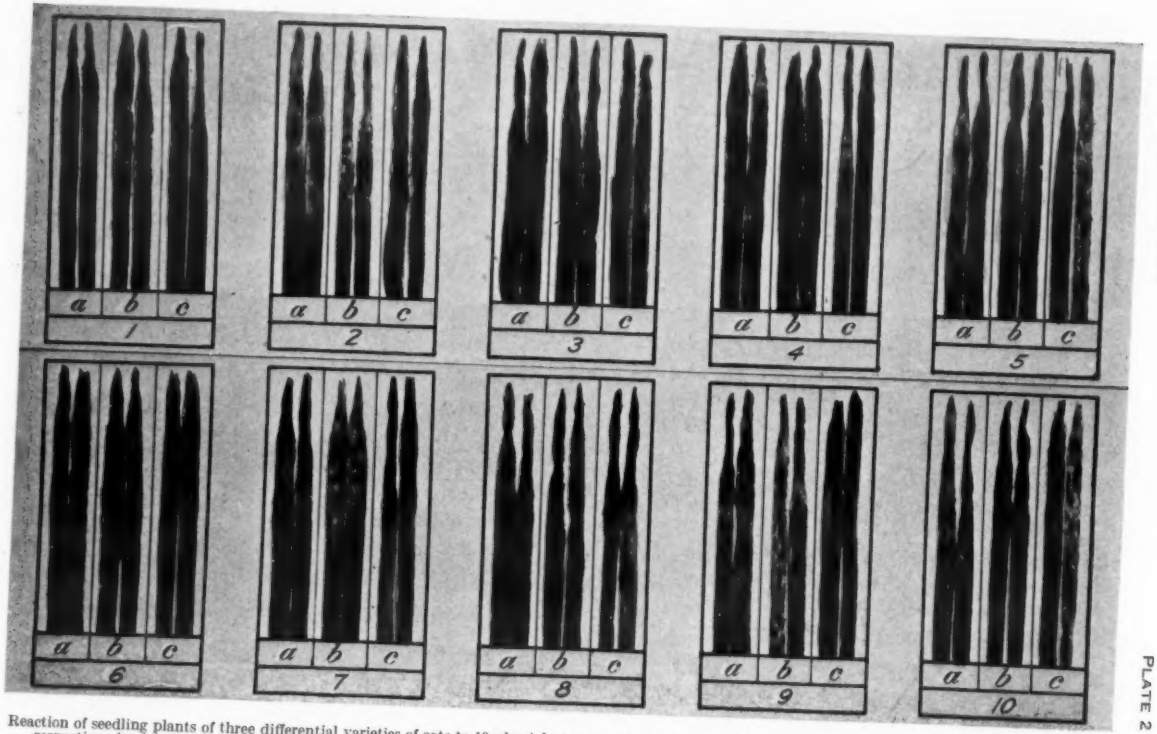
The oat varieties of the present study, tested both in the adult and seedling stages, were as follows: Gopher (C. I.[4] 2027), Victoria (C. I. 2401), Minnesota 742 (C. I. 2874)[5], Anthony (C. I. 2143), Minrus (C. I. 2144), Rainbow (C. I. 2345), and Richland (C. I. 787). These oat strains were used because of their importance in the breeding project of the Minnesota Agricultural Experiment Station or because of differential characteristics valuable in the identification of stem rust races. The varieties Gopher and Victoria always have been highly

[4] C. I. refers to accession number of the Division of Cereal Crops and Diseases (formerly Office of Cereal Investigations).

[5] Hybrid selection, Minnesota nursery stock no. II-22-220.



Reaction classes assigned to differential varieties, based on infection types produced by physiologic races of stem rust on seedling plants of oats: *A*, Resistant class, exemplified by type *0* (no infection at all or pronounced necrotic flecks), type 1 (minute uredia surrounded by distinct necrotic areas), and type 2 (small uredia embedded in hypersensitive areas ranging from slight necrosis to definite chlorosis); *B*, susceptible class, portrayed by type 3 (medium size uredia with some chlorosis but no necrosis), and type 4 (large and confluent uredia resulting in severe infection); C, mesothetic class, marked by type X (infection heterogeneous and rather ill-defined).



Reaction of seedling plants of three differential varieties of oats to 10 physiologic races of *Puccinia graminis avenae*. Arabic numerals denote key numbers of respective physiologic races. The differential varieties represented are designated as follows: (*a*), White Tartar, (*b*), Richland, (*c*), Sevnothree. (Compare illustrations with results given in table 1.)

susceptible to all known physiologic races of *Puccinia graminis avenae*. Anthony, Minrus, and Minnesota 742 react very much like their White Tartar (White Russian) parent to the various rust races. The stem rust reactions of Rainbow and Richland are virtually identical, and either may serve as a substitute differential host for the other.

Gopher, a pure-line selection from Sixty-Day, was described in detail by Stanton, Griffee, and Etheridge (*35*). Victoria was described by Murphy and Stanton (*23*) and again by Stanton and Murphy (*36*). Minnesota 742 is a strain that was obtained from crossing Minota×White Tartar by Black Mesdag; it is generally extremely resistant to loose and covered smut, and is at least moderately resistant to most parasitic races of stem rust. Minrus was described by Wilson and Arny (*42*) as Minnesota 693. Anthony and Rainbow were described by Stanton, Gaines, and Love (*34*). Richland was described by Burnett, Stanton, and Warburton (*4*).

In addition to the seven varieties just enumerated, six others were tested in the seedling stage to all known parasitic races of *Puccinia graminis avenae*, i. e., races 1 to 10, inclusive, and in the adult stage to race 6 alone. Fourteen more varieties were tested to all 10 races, but only in the seedling stage. A key for the identification of these rust races follows.

Analytical key for the identification of physiologic races of Puccinia graminis avenae on the basis of their pathogenicity on three selected differential varieties within the genus Avena

Reaction of differential hosts:	*Physiologic race (key no.)*
White Tartar resistant:	
Richland resistant:	
Sevnothree resistant	1
Sevnothree mesothetic	5
Sevnothree susceptible	2
Richland mesothetic	9
Richland susceptible:	
Sevnothree mesothetic	10
Sevnothree susceptible	8
White Tartar susceptible:	
Richland resistant:	
Sevnothree resistant	3
Sevnothree susceptible	7
Richland susceptible:	
Sevnothree resistant	4
Sevnothree susceptible	6

The various types of infection that the oat stem rust fungus is capable of producing under different circumstances are illustrated in plate 1. The parasitic behavior of the several rust races, cultured at a temperature fluctuating around 70° F., is recorded in table 1. The reactions of seedlings of three differential varieties of oats to each of the 10 physiologic races are depicted in plate 2.

respective physiologic races. The differential varieties represented are designated as follows: (*a*), White Tartar, (*b*), Richland, (*c*), Sevnothree. (Compare illustrations with results given in table 1.)

TABLE 1.—*Reaction of differential varieties of Avena spp., in the seedling stage, to physiologic races of Puccinia graminis avenae, expressed in ranges and means of infection types*

Physiologic race (key no.)	Reaction of differential hosts [1]								
	White Tartar			Richland			Sevnothree		
	Range		Mean	Range		Mean	Range		Mean
	Minimum	Maximum		Minimum	Maximum		Minimum	Maximum	
1	2−	3n+	2+	0;	1++	1	1−	2++	1±
2	2=	3n+	2++	1−	2+	1+	4=	4+	4
3	3	4+	4−	1	2++	1	0;	1	1−
4	4−	4++	4	4−	4++	4+	0;	1+	1
5	1++	3n+	2++	1−	2+	1++	1c	X++	X±
6	4+	4++	4+	4+	4++	4+	4+	4++	4+
7	3	4+	4	0;	2+	2=	3	4+	4−
8	0;	2+	2	3	4+	4	3	4	3++
9	2=	3n+	2±	X=	4n+	X+	3++	4++	4
10	1	3+	2	3	4++	4	X−	X++	X+

[1] 0, practically immune (no infection whatever or pronounced necrotic flecks or lesions); 1, extremely resistant (minute uredia surrounded by solid necrotic areas); 2, moderately resistant (uredia small with hypersensitive areas varying from sharp necrosis to pronounced chlorosis); 3, moderately susceptible (medium-size uredia with slight chlorosis but no necrosis); 4, extremely susceptible (large confluent uredia resulting in very severe infection); X, characteristically mesothetic (infection heterogeneous and ill-defined). Plus and minus signs indicate a slightly greater or smaller amount of rust within a given infection type; the sign of equality denotes double minus. Necrotic flecks are designated by a semicolon; n = distinct necrosis; c = apparent chlorosis.

Races 1 to 5, inclusive, were originally described by Stakman, Levine, and Bailey (*31*), and later elaborated on by the last-named author (*2*). Further studies with these same races, as well as races 6 to 9, inclusive, were reported in detail by Gordon (*9*). Race 10 was first discovered and described by Cotter (*5*). The pathogenic behavior of all these races was summarized by Stakman et al. (*32*). A few of the races are widely distributed and of common occurrence, some are extremely virulent, others are relatively innocuous.

The procedure for testing the different oat varieties in the seedling stage was, except for some minor modifications, essentially the same as that described by Stakman, Levine, and Bailey (*31*). The method of testing the adult plants was as follows: Of the seven varieties previously listed, all but Richland were planted in three sets, sown 3 days apart. Each set consisted of thirty 4-inch pots, making 90 pots for each variety. After the pots had been steamed for half an hour at 12 pounds pressure, they were filled with soil steamed for 2½ hours. Three to five seeds of a given variety were sown in each pot. When the plants reached the early seedling stage, they were thinned to a single plant per pot and allowed to grow to a late heading stage.

All potted plants were set on benches in a greenhouse where for some time previous there had been no stem rust cultured. Here the plants grew under natural light conditions, which in the course of the experiment were unusually favorable. The atmospheric temperature in the greenhouse fluctuated between 67° and 80° F. The pots were at first placed close together; as the plants became larger, these pots were arranged in double rows, allowing a 6-inch space between pairs. When the plants attained a height of 8 inches, a cord lattice was made to hold them erect. When they were approximately 20 inches tall,

the cord support was removed and in its stead individual wire supports for each plant were substituted.

The plants were watered as frequently as necessary to prevent severe wilting, usually every 24 hours. The growth produced was somewhat more succulent and tender than that usually attained by plants grown in the field. What effect, if any, such succulence had on the reaction of the plants to the rust attack was not experimentally determined, except as manifested by the character of the rust development and the resultant infection types.

Eight days before a given set of adult plants was inoculated, seed of corresponding varieties was sown in 4-inch pots to provide seedlings for simultaneous inoculation with the respective physiologic races. At the time of inoculation, the adult plants were 30 to 40 inches tall and in the boot stage. There were one to five tillers per plant, all plants appearing vigorous and showing no indication of chlorosis or other weakness. Immediately preceding inoculation, the potted adult plants were placed in tall metal cylinders, which rested in pans containing water to a depth of 1½ to 2 inches. Each incubation chamber contained 20 plants of two separate oat varieties or strains.

The different sets of oat plants were inoculated seriatim with urediospores of a particular race in the following manner: The adult plants were sprayed with tap water and thoroughly brushed with rusted seedling plants of Victory oats severely infected by a known rust race that had been carefully tested for purity. The moist chambers then were covered with glass panes. Three days later the inoculated plants were removed from the incubators and placed on greenhouse benches. Seedling plants were inoculated at the same time and incubated for 2 days instead of 3. Incubation conditions were comparable for the different oat sets and growth stages. This method was used for all physiologic races except race 10, which was not available at the time. Subsequently, race 10 also was used to test the reaction of seedling and adult plants of the seven oat varieties under uniform cultural conditions. The variety Richland was tested as a unit to each of the first nine physiologic races, but was included with the other six varieties when their reaction to race 10 was determined. A special test of the reaction of adult plants to race 6 was made with six additional varieties, viz, Hajira (C. I. 1001), Iogold (C. I. 2329), Joanette strain (C. I. 2660), Sevnothree (C. I. 3251), Victory (C. I. 1145), and White Tartar (C. I. 551).

Rust notes were taken on each of the sets 17 days after their respective inoculations. At that time pustules on both seedlings and maturing plants had reached their optimum development. Although the infection varied with the different races on the several varieties, it was generally rather severe on the susceptible ones. The reaction of all varieties and strains, even when resistant, was clear-cut. Three reaction classes were recognized, namely, resistant, mesothetic, and susceptible. These were designated in the records by the capital letters R, M, and S, respectively.

As may be seen from plate 1, the resistant class embraces infection types 0, 1, and 2 (type 0 indicating immunity, type 1 high resistance, and type 2 moderate resistance); the mesothetic class includes but a single infection type, X, indicating a heterogeneous and ill-defined, somewhat intermediate reaction; and the susceptible class is represented by types 3 and 4 (type 3 standing for moderate and type 4

for complete susceptibility). In this study, rust observations on seedlings are recorded in terms of detailed infection types, whereas readings on adult plants are recorded in terms of general reaction classes. The respective symbols are used to denote each. Wherever necessary, plus and minus signs are used to show greater or smaller fluctuations within a norm.

RESULTS OF INVESTIGATION

The results obtained from the tests with seedlings and adult plants of the different oat varieties are summarized in tables 2 and 3 and illustrated in plates 3 to 8, inclusive. The occurrence of physiologic races of oat stem rust [6] and their relative prevalence in the United States are recorded in tables 4 and 5.

TABLE 2.—*Average infection produced by 10 physiologic races of Puccinia graminis avenae on seedlings of 27 varieties and strains of oats grown under greenhouse conditions and constituting four distinct reaction, or differential, groups*

Variety tested			Mean infection type produced by physiologic race (key no.)—									
Reaction group no.	Name	C. I. no.	1	2	3	4	5	6	7	8	9	10
1	Anthony	2143	2=	2−	4+	4+	2−	4−	4	2−	2−	2±
	Green Mountain	1892	2	2−	4	4+	2±	4	4−	2	2+	2
	Minnesota 742	2874	2=	2−	4	4+	1±	4−	4+	2−	1=	2−
	Minrus	2144	2−	2=	4+	4+	2−	4±	4±	2−	2±	2±
	White Tartar	551	2±	2−	4+	4+	2−	4±	4+	2−	2=	2±
2	Hajira	1001	1−	1−	1+	4	1	4±	2=	4	X±	4±
	Hawkeye	2464	2=	1±	2=	4−	2	4−	2	4−	X+	4+
	Iogold	2329	1	1−	1±	4+	1−	4+	1+	4+	X+	4+
	Iowa D67	2870	1−	1+	2=	4+	2±	4+	2+	4	X=	------
	Rainbow	2345	1−	1±	2−	4+	1	4±	1+	4±	X+	4=
	Richland	787	1±	1+	1+	4±	1±	4±	1±	4±	X±	4−
3	Joanette strain	2460	1±	4+	1−	1−	X	4+	4±	4+	4+	X
	Sevnothree	3251	1	4	1−	1±	X±	4±	4−	4=	4	X±
	Alber	2766	4−	4+	4±	4+	4+	4−	4	4+	4+	4+
	Belar	2760	4	4−	4	4−	4−	4	4	4	4	4+
	Bond	2733	4±	4	4=	4+	4−	4+	4±	4+	4+	4
	Cassel	2911	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
	Cowra	2761	3+	4	4	4±	4+	4	4+	4=	4	4+
	Fulmer	2912	4=	4−	4	4	4+	4	4+	4	4	4+
4	Glabrota	2630	3+	4	4	3+	4=	4	4	4	4	4+
	Gopher	2027	4=	4	4+	4+	4	4+	4	4±	4±	4
	Kareela	2774	4	4±	4	4−	4+	4+	4±	4+	4+	------
	Ruakura	2025	4+	4±	4+	4+	4+	4+	4	4+	4+	4+
	Sterisel	2891	4	4±	4	4+	4	4+	4	4	4+	4+
	Swedish Select	134	4+	4	4+	4+	4+	4+	4	4	4−	4
	Victoria	2401	4+	4±	4	4	4+	4+	4±	4+	4+	4+
	Victory	1145	4+	4±	4	4±	4+	4+	4+	4+	4+	4±

[6] In conformity with the decision of the Sixth International Botanical Congress, the designations "parasitic and/or physiologic race or races" are given preference in this paper over their former equivalents, "biologic and/or physiologic form or forms."

TABLE 3.—*Comparative reaction under greenhouse conditions of seedling and adult plants of seven varieties and strains of oats to 10 different physiologic races of stem rust (Puccinia graminis avenae)*

Physiologic race (key no.)	Inoculation results on varieties tested [1]													
	A				B						C			
	Rainbow		Richland		Anthony		Minnesota 742		Minrus		Gopher		Victoria	
	Seedlings	Adults	Seedlings	Adults	Seedlings	Adults	Seedlings	Adults	Seedlings	Adults	Seedlings	Adults	Seedlings	Adults
1	1	R	1	R	2	R	2	R	2	R	3	S	4	S
2	1	R	1	R	2	R	2	R	2	R	4	S	4	S
3	1	R	1	R	4	S	4	S	4	S	3	S	4	S
4	4	S	4	S	4	S	4	S	4	S	4	S	4	S
5	1	R	1	R	2	R	1	R	2	R	4	S	4	S
6	4	S	4	S	4	S	4	S	4	S	4	S	4	S
7	1	R	1	R	4	S	4	S	4	S	4	S	4	S
8	4	S	4	S	2	R	2	R	2	R	4	S	4	S
9	X	M	X	M	2	R	1	R	2	R	4	S	4	S
10	4	S	4	S	2	R	2	R	2	R	4	S	4	S

[1] The several varieties are grouped into three categories, A, B, and C, according to their reaction to the various physiologic races. For seedling plants the average infection type is given; for adult plants, the reaction class, as follows: R, resistant; S, susceptible; and M, mesothetic or intermediate in reaction.

TABLE 4.—*Summary of the distribution of physiologic races of Puccinia graminis avenae obtained from various parts of the United States during the 15-year period 1921–35, arranged according to standard geographic divisions and oat-growing areas*

Place of origin	Total of isolates	Distribution of physiologic race (key no.)—									
		1		2		5		7		10	
	Number	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Geographic divisions:											
Atlantic States	31	2	6.5	20	64.5	7	22.6	1	3.2	1	3.2
East North Central States	331	7	2.1	211	63.8	111	33.5	.....	.....	2	.6
West North Central States	965	12	1.2	703	72.9	249	25.8	1	.1	.....	.....
South Central States	375	8	2.1	260	69.3	107	28.6	.....	.....	.....	.....
Western States	43	1	2.3	30	69.8	12	27.9	.....	.....	.....	.....
Entire country	1,745	30	1.7	1,224	70.1	486	27.9	2	.1	3	.2
Oat-growing areas:											
Great Lakes:											
Ohio	84	.....	.....	56	66.7	28	33.3	.....	.....	.....	.....
Indiana	27	3	11.1	14	51.9	10	37.0	.....	.....	.....	.....
Illinois	60	1	1.7	39	65.0	20	33.3	.....	.....	.....	.....
Michigan	55	2	3.7	35	63.6	18	32.7	.....	.....	.....	.....
Wisconsin	105	1	1.0	67	63.8	35	33.3	.....	.....	2	1.9
Total	331	7	2.1	211	63.8	111	33.5	.....	.....	2	.6
Mississippi Valley:											
Minnesota	306	7	2.3	218	71.3	80	26.1	1	.3	.....	.....
Iowa	126	2	1.6	87	69.0	37	29.4	.....	.....	.....	.....
Missouri	49	.....	.....	38	77.6	11	22.4	.....	.....	.....	.....
Total	481	9	1.9	343	71.3	128	26.6	1	.2	.....	.....
Great Plains:											
North Dakota	145	2	1.4	93	64.1	50	34.5	.....	.....	.....	.....
South Dakota	119	.....	.....	86	72.3	33	27.7	.....	.....	.....	.....
Nebraska	77	.....	.....	59	76.6	18	23.4	.....	.....	.....	.....
Kansas	143	1	.7	122	85.3	20	14.0	.....	.....	.....	.....
Oklahoma	125	2	1.6	103	82.4	20	16.0	.....	.....	.....	.....
Texas	241	6	2.5	149	61.8	86	35.7	.....	.....	.....	.....
Total	850	11	1.3	612	72.0	227	26.7	.....	.....	.....	.....
Entire region	1,662	27	1.6	1,166	70.2	466	28.0	1	<.1	2	>.1

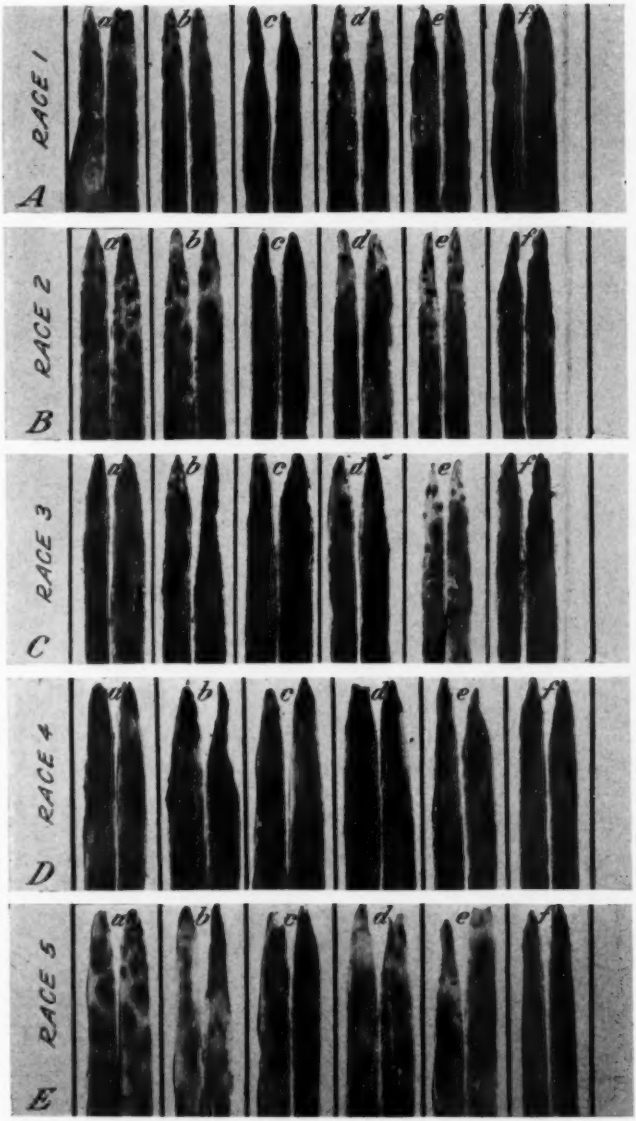
TABLE 5.—*Frequency of occurrence of physiologic races of Puccinia graminis avenae isolated from rusted field specimens obtained from different parts of the United States during the various years of the 15-year period 1921–35, inclusive*

Years	Total of isolates	Distribution of physiologic race (key no.)—									
		1		2		5		7		10	
	Number	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
First 5 years:											
1921	12	5	41.7	7	58.3						
1922	12	3	25.0	6	50.0	3	25.0				
1923	13	7	53.8	5	38.5	1	7.7				
1924	51	1	2.0	30	58.8	20	39.2				
1925	29	2	6.9	27	93.1						
Total	117	18	15.4	75	64.1	24	20.5				
Second 5 years:											
1926	61	6	9.8	53	86.9	2	3.3				
1927	101			90	89.1	11	10.9				
1928	256			208	81.3	48	18.7				
1929	224	1	.4	155	69.2	68	30.4				
1930	144	1	.7	84	58.3	57	39.6			2	1.4
Total	786	8	1.0	590	75.1	186	23.7			2	.2
Third 5 years:											
1931	387	1	.3	260	67.2	126	32.5				
1932	108	1	.9	58	53.7	49	45.4				
1933	58	1	1.7	37	63.8	17	29.3	2	3.5	1	1.7
1934	130			99	76.2	31	23.8				
1935	159	1	.6	105	66.1	53	33.3				
Total	842	4	.5	559	66.4	276	32.8	2	.2	1	.1
Entire period	1,745	30	1.7	1,224	70.1	486	27.9	2	.1	3	.2

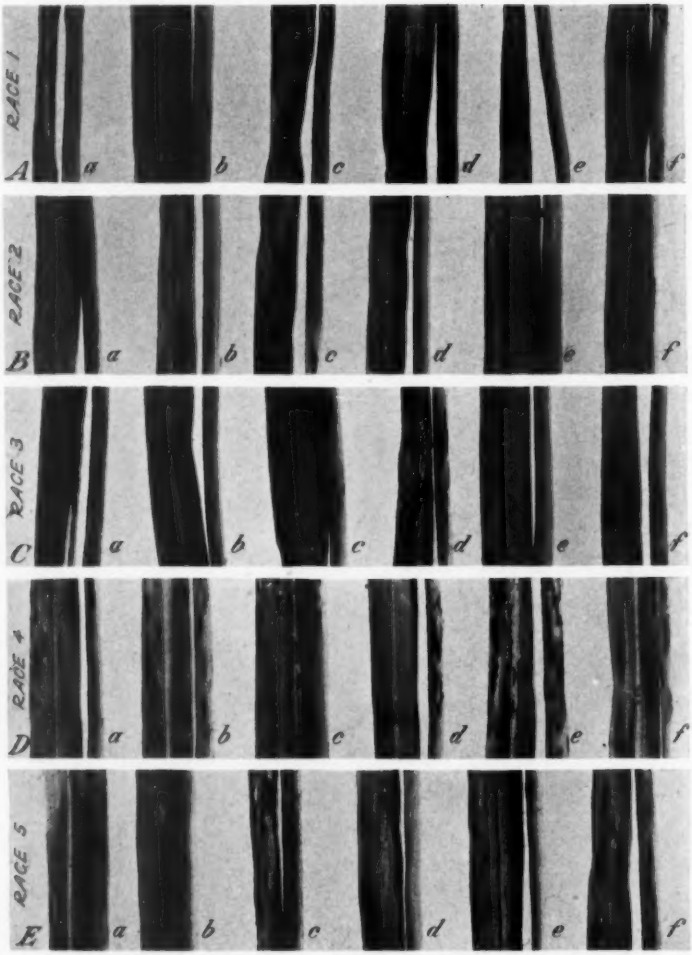
SEEDLING AND ADULT PLANT REACTION OF OAT VARIETIES

In table 2 are listed 27 varieties and strains of oats that were tested in the seedling stage, under fairly uniform greenhouse conditions, to all of the physiologic races of *Puccinia graminis avenae* known to date. It will be noted that on the basis of their reaction to the various rust races, these varieties were readily grouped into four categories. In the first three of these are included varieties possessing differential reaction characteristics that make possible the identification of physiologic races of the oat stem rust fungus. The varieties included in the fourth group show very little difference in their reactions, either with respect to each other or to the several physiologic races. Consequently, they are of no differential value in the determination of physiologic races; nor are they of parental value in breeding for stem rust resistance, although they may possess other desirable features.

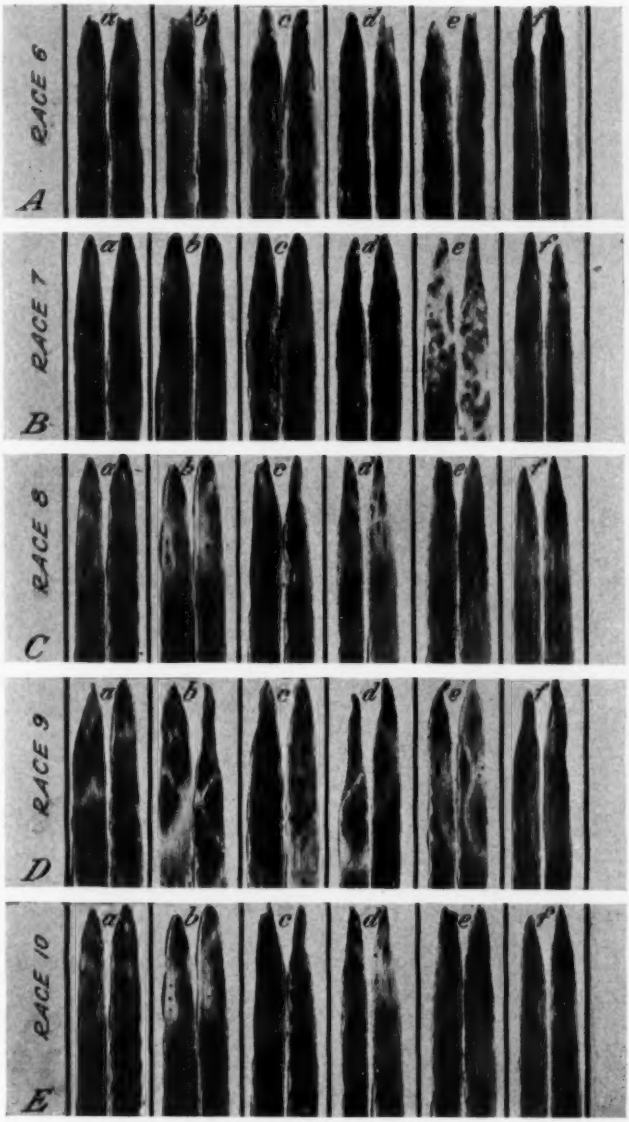
The three groups containing varieties with differential traits are listed in the order in which the standard differentials appear in the analytical key (p. 717) and infection record (table 1). The varieties within each group are arranged, for the sake of convenience, in alphabetical order. Incidentally, the last variety in each of these groups happens to be the standard differential host now used in the identification of physiologic races of *Puccinia graminis avenae*. The types of infection usually produced by these races on the differential varieties—White Tartar, Richland, and Sevnothree—are illustrated in plate 2. The origin of Sevnothree (strain 703) is described in detail by Bailey (*2*); reference to Richland has already been made; White Tartar is discussed by Levine, Stakman, and Stanton (*20*).



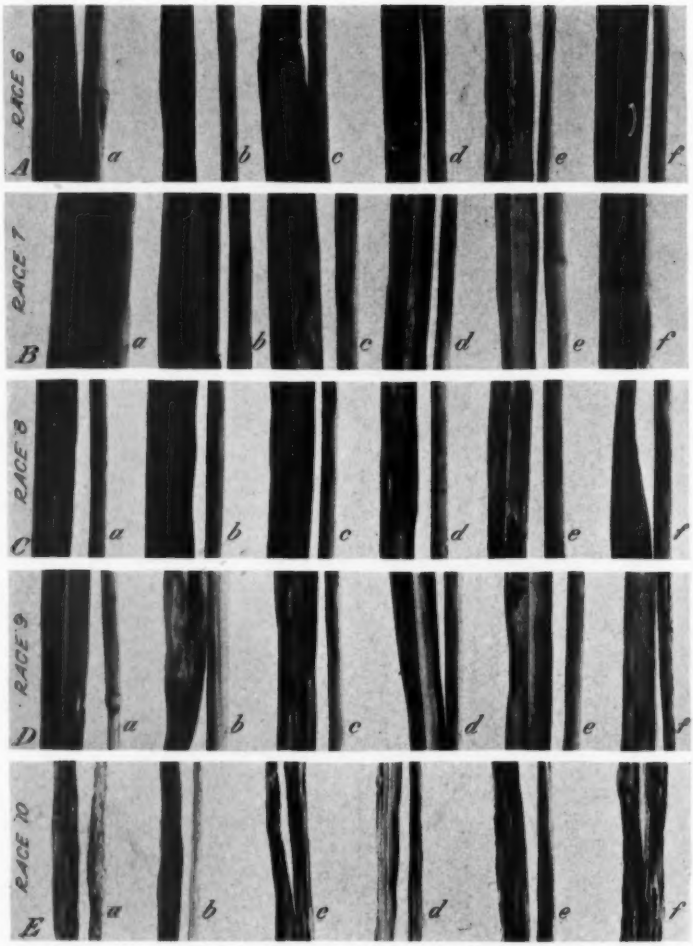
Reaction of seedling plants of six oat varieties—(*a*) Anthony, (*b*) Minn. 742, (*c*) Gopher, (*d*) Minrus, (*e*) Rainbow, and (*f*) Victoria—to five physiologic races of *Puccinia graminis avenae* (races 1 to 5, respectively). (Compare with reaction of adult plants of corresponding varieties, illustrated in plate 4.)



Reaction of adult plants of six oat varieties—(*a*) Anthony, (*b*) Minn. 742, (*c*) Gopher, (*d*) Minrus, (*e*) Rainbow, and (*f*) Victoria—to five physiologic races of *Puccinia graminis avenae* (races 1 to 5, respectively). (Compare with reaction of corresponding seedling plants, illustrated in plate 3.)



Reaction of seedling plants of six oat varieties—(*a*) Anthony, (*b*) Minn. 742, (*c*) Gopher, (*d*) Minrus, (*e*) Rainbow, and (*f*) Victoria—to five physiologic races of *Puccinia graminis avenae* (races 6 to 10, respectively). (Compare with reaction of adult plants of corresponding varieties, illustrated in plate 6.)



Reaction of adult plants of six oat varieties—(*a*) Anthony, (*b*) Minn. 742, (*c*) Gopher, (*d*) Minrus, (*e*) Rainbow, and (*f*) Victoria—to five physiologic races of *Puccinia graminis avenae* (races 6 to 10, respectively) (Compare with reaction of corresponding seedling plants, illustrated in plate 5.)

There are five varieties included in group 1. They either have side panicles or are the progeny of crosses in which a "side-oats" variety was one of the parents. They are all midseason or late in maturity. Each variety in this group is moderately resistant to six rust races (key nos. 1, 2, 5, 8, 9, and 10) and highly susceptible to the other four (key nos. 3, 4, 6, and 7). Group 2 consists of six varieties. All of these are common oats; they have yellow lemmas, and all but one are early maturing. They are, on the whole, very resistant to five races (key nos. 1, 2, 3, 5, and 7), mesothetic to one race (key no. 9), and very susceptible to four races (key nos. 4, 6, 8, and 10). In group 3 there are only two varieties. Both are pure-line selections from varieties possessing black lemmas and midseason maturity. They are extremely resistant to three parasitic races (key nos. 1, 3, and 4), typically mesothetic to two races (key nos. 5 and 10), and completely susceptible to five races (key nos. 2, 6, 7, 8, and 9).

The classification of the above oat groups is based on the infection types produced by the different stem rust races, cultured under favorable light intensities and at a prevailing atmospheric temperature of about 70° F. The reaction of the varieties in groups 1, 2, and 4 are but slightly affected by temperature and light variations, except in the case of race 9, where group 2 is concerned. Group 3, on the other hand, is very sensitive to changes in light and especially so to changes in temperature in the case of races 5 and 10. According to Gordon (*9*), Joanette strain loses its high resistance to races 1, 3, and 4 when cultured at high temperatures. Neither light nor temperature, however, seems to affect appreciably the susceptibility of Joanette strain and Sevnothree to the remaining five races.

The results summarized in table 3 and illustrated in plates 3 to 6, inclusive, reveal apparently complete agreement between the reaction of the seedlings of a given variety and that of the adult plants of the same variety. This correlation was not restricted to reaction to any one physiologic race, but held good for all of them; nor was it limited to any one oat variety, but prevailed for all seven varieties tested.

Plants of varieties on the seedlings of which a certain physiologic race produced an infection classified as type 1 or type 2 were invariably resistant in the adult stage also to that same race, and were designated by the symbol R. Though the distinction between infection types 1 and 2 was quite clear on seedling plants, corresponding adult plants could not be so readily differentiated according to infection type; therefore, the reaction class was used as the criterion for classification in the case of adult plants. The same held true to a great extent also in the case of susceptible reactions. Thus, plants on which infection types 3 and 4 developed in the seedling stage were always either moderately or wholly susceptible in the adult stage and were characterized by the symbol S. Although the infection produced by race 9 on adult plants of Rainbow and Richland was not typically heterogeneous, the reaction was more nearly mesothetic than otherwise, thus justifying its designation by the symbol M.

The varieties Rainbow and Richland reacted alike to all 10 rust races and were highly resistant in the adult as well as the seedling stage to 5 of these races. Anthony, Minnesota 742, and Minrus reacted very much the same to all races and were at least moderately resistant to six of them. Gopher and Victory were about equally

susceptible to all races. (Compare illustrations in plates 3 and 5 with those in plates 4 and 6, respectively, for corresponding results.)

Judging solely by the reaction of the 7 varieties just discussed, the 10 existing races could not be distinguished as so many units; instead, some of them could be combined in composite groups, as follows: Composite I, consisting of races 1, 2, and 5; composite II, embracing races 3 and 7; composite III, including races 4 and 6; and composite IV, consisting of races 8 and 10; with race 9 occupying an intermediate position between composites I and IV. This situation, obviously, is due to the fact that no representatives of oat group III, listed in table 2, were included among the seven varieties used in the maturative test. But essentially the same race combinations might be made even when Joanette strain or Sevnothree is used, as in the seedling experiment, if proper allowance is made for the somewhat erratic behavior of these varieties. Race 9 might be readily considered a counterpart of either race 8 or race 10, and thus a member of composite IV.

The results obtained in this study seem not to indicate any weakening in the resistance of the adult plants because of their succulent condition. While such a variety as Richland was highly susceptible in the adult stage to races 4, 6, 8, and 10 (pl. 7), it was extremely resistant to the other races. Considering expected reactions, the same was true of the other varieties. The reaction in each case depended on whether a physiologic race was virulent or innocuous on a particular oat variety. Race 6 is by far the most virulent of the lot,[7] causing severe infection on all varieties tested, seedlings and adult plants alike. The varieties depicted in plate 8 belong to all four oat groups, yet they are similarly affected by the rust of race 6. Races 4, 7, and 8 are next in order of virulence. These are followed by races 9 and 10, which in turn are followed by races 2 and 3. Then comes race 5, with race 1 last in order of virulence.

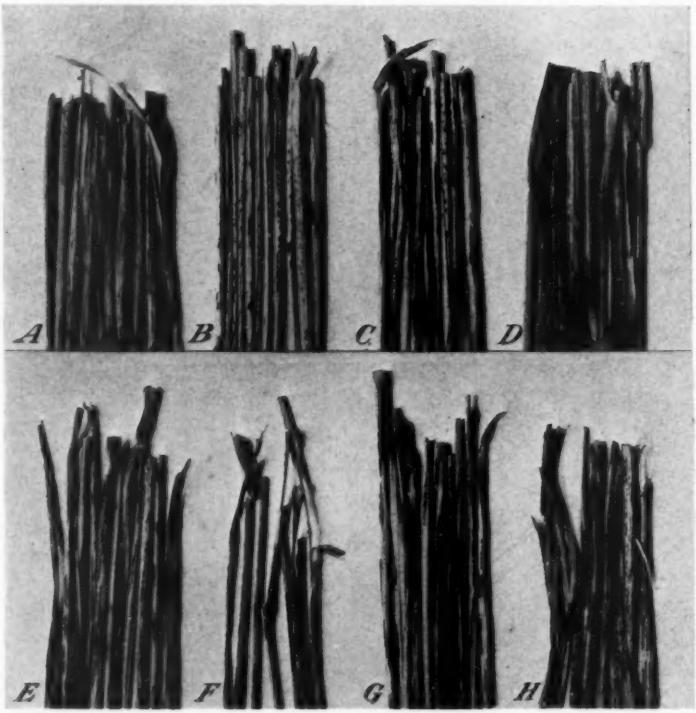
PREVALENCE AND DISTRIBUTION OF PHYSIOLOGIC RACES OF STEM RUST

In view of the results obtained with regard to the relative susceptibility or resistance of oat plants at different stages of their development, the question of practical importance is "How many of the known races of *Puccinia graminis avenae*, and precisely which of them, exist in the United States, and what is their geographic distribution and frequency of occurrence?" A survey conducted for the past 15 years may afford an answer to this query.

An extensive study of the physiologic specialization of oat stem rust has been carried on at University Farm, St. Paul, Minn., since 1921. Although specimens from different parts of the world were identified, major attention was paid to field collections obtained from various sections of the United States. These were cultured in the greenhouse on the standard differential varieties, and their identity was determined with the aid of the analytical key (p. 717) and verified by means of the infection record (table 1). In all, five separate physiologic races have thus far been found to be present in variable frequency in this country. Since the identification was not carried out under strictly controlled environmental conditions, it is not impossible that other forms remained undetected.

[7] Since this manuscript was submitted for publication, Welsh (*41*) has reported securing a few oat lines from a cross of Hajira Strain × Joanette strain possessing greater resistance than either parent to several physiologic races of *Puccinia graminis avenae*, including race 6.

Relatively high and rather uniform susceptibility of adult plants of Richland oats to four physiologic races of *Puccinia graminis avenae*: *A*, Race 4; *B*, race 6; *C*, race 8; *D*, race 10. (Compare these infections with those produced by the same races on Richland seedlings, as illustrated in plate 2.)



Severe infection produced by physiologic race 6 of *Puccinia graminis avenae* on adult plants of eight varieties of oats, viz.: *A*, Anthony; *B*, Hajira; *C*, Iogold; *D*, Joanette strain; *E*, Richland; *F*, Sevnothree; *G*, Victory; *H*, White Tartar.

The races identified under the conditions of the experiments were key nos. 1, 2, 5, 7, and 10. Summaries of their distribution by geographic divisions and crop areas during the 15-year period under review, and their annual occurrence in the country as a whole are presented in tables 4 and 5, respectively. In the course of the entire 15 years from 1921 to 1935, altogether 1,745 isolations were identified. Only five of these, or 0.3 percent, constituted the total for races 7 and 10 combined.

The relative proportion of the number of times these two races were isolated to the total number of isolations made during the 15-year period is so insignificant, and the circumstances of their incidence of such a nature, that it appears doubtful whether either of them will soon, if ever, become a potent factor in the stem rust epidemics on oats in the United States. Race 7 thus far has been found only twice in this country: once, near rusted barberries at Presque Isle, Maine; and the second time, in the rust nursery at St. Paul, Minn. Race 10 was isolated three times: twice from specimens collected in the neighborhood of infected barberries at Jefferson, Wis.; and once, under like conditions, at Presque Isle, Maine.

Of the remaining three races, the least common for the period as a whole was race 1, which so far has been isolated but 30 times in the entire United States, constituting only 1.7 percent of all isolations made. Race 2 comprised 70.1 percent of the total and, on an average, was almost exactly two and one-half times as common as race 5, which constituted 27.9 percent of the total. There were no very great differences in the relative strength of these races in the several geographic divisions and crop areas, although considerable variation did occur between different States in the oat-growing region. Thus, Kansas furnished the highest proportion of race 2 (85.3 percent), while Indiana yielded the lowest (51.9 percent), taking the whole 15-year period into consideration.

A preponderant majority of the isolates (1,662 out of 1,745, or 95.24 percent) came from the oat-growing region. Of these, more than half (850) were obtained from the Great Plains area; the upper Mississippi Valley provided 481 isolates; and the Great Lakes area yielded 331. Minnesota furnished the highest number for a single State, while the fewest came from Indiana. Further details may be found in table 4.

It may be seen from table 5 that not only was there an annual fluctuation in the relative proportion of the three dominant races of *Puccinia graminis avenae* in the rust-survey territory of the United States but also there appeared to be a definite trend in the population relationships of these races, as determined by quinquennial computations. While throughout, except in 1 year, race 2 was invariably in the forefront, race 5, when present, was, with but two exceptions, in second place, and race 1, for the most part trailing behind, was at the head of the list once, in 1923. However, that was one of the years when the number of isolates obtained was very limited, and the apparent predominance of race 1 may not be significant.

In the first quinquennium (1921–25), race 1 occupied third position but was a close rival of race 5, and with 15.4 percent of the total number of isolates, seemingly was not a negligible factor in the oat stem rust epidemics of that period. However, it soon after all but disappeared, for in the second quinquennium (1926–30) it composed but

1.0 percent of the total, and in the third quinquennium (1931–35) it had dwindled to a mere 0.5 percent.

Race 2 follows a zigzag course, ranging from 38.5 to 93.1 percent of the respective annual totals. Considering the situation by 5-year periods, race 2 traced a rather gentle curve—64.1 percent in the first quinquennium, 75.1 percent in the second, and 66.4 percent in the third.

It is race 5 that shows a gradual and consistent quinquennial rise, from 20.5 to 23.7 to 32.8 percent of the respective 5-year totals. But this race, too, suffers from considerable annual variability, being entirely absent in 2 years and reaching 45.4 percent in at least 1 year.

It may be pertinent to observe at this juncture that, from a rather limited survey of the stem rust situation in Mexico, it appears that only races 2 and 5 are present in that country, and that up to this time they have occurred there in a 5:3 ratio. According to the results published by Bailey (*2*), including his data appearing in the report of the Dominion botanist (*3*), and by Gordon (*9*), the three races 1, 2, and 5 constituted 96.7 percent of the 1,342 isolations made by those authors from Canadian collections during the 10-year period 1921–30. The remaining 3.3 percent consisted of the other known races exclusive of race 10. Waterhouse (*39*) reports that in Australia races 1 and 2 together constituted over 88 percent of the 371 isolates of oat stem rust identified from 1925 to 1933, inclusive. Other races found there were 3, 6, and 7, the latter making up 8.1 percent of the total. A few isolations from New Zealand collections made by this authority yielded only races 1 "and/or" 2. From the results published by Verwoerd (*38*) it appears that races 3 and 7 occur in South Africa, while Stakman, Levine, and Bailey (*31*) had previously identified races 2 and 3 from material they received from that country. Tedin (*37*) noted the occurrence in Sweden of races 3, 4, 6, 7, and 8, but none of the prevalent American races. The present writers isolated races 2, 6, and 7 from uredial collections received from Palestine; and races 2, 5, and 7 from specimens obtained from New Zealand.

It is evident from the results recorded in this paper that the oat stem rust epidemics in the United States during the 15-year period 1921–35 were almost entirely due to the widespread distribution and common occurrence of races 2 and 5, the two races combined constituting 98 percent of the total number of isolates. This study has also brought out the fact that so far the physiologic specialization of *Puccinia graminis avenae* in this country has been rather definitely restricted.

SUMMARY AND CONCLUSIONS

Inoculations with the 10 known physiologic races of oat stem rust, *Puccinia graminis avenae*, were made under comparable and reasonably uniform greenhouse conditions on 27 varieties and strains of oats in the seedling stage; on 7 of these varieties in the adult stage, also with all 10 races; and on 6 more of the varieties and strains in the adult stage, with race 6 only.

Special care was taken to assure the purity of the physiologic races used. For this purpose periodic checks were made of the behavior of each race on the standard differential varieties grown under similar light intensities and fairly well-controlled temperatures. Assurance also was provided that the differential hosts themselves were free

from admixture. This was accomplished by the use of established pure rust races as biological reagents.

The differences in the reactions of the 27 oat varieties in the seedling stage made it possible to group them in 4 categories. In three of these were included the varieties that possess reaction characteristics typical of differential hosts, enabling their use as substitutes in the identification of physiologic races of *Puccinia graminis avenae*. The fourth group consisted of those varieties showing no discernible difference in their reactions, whether with respect to each other or to the different physiologic races.

There was a remarkably close agreement in the reactions of seedling and maturing plants of every one of the varieties and strains tested, regardless of parasitic race involved; in no case was a variety resistant or susceptible as a seedling or adult plant without a corresponding reaction in the other growth phase tested. It would seem from these results that seedling reaction is a reliable index of the reaction of adult oat plants to specific physiologic races of oat stem rust. It would be desirable, however, to investigate this problem further before drawing too general conclusions, since important exceptions, not considered within the limits of these experiments, may exist.

While during the 15-year period 1921–35, five different physiologic races (1, 2, 5, 7, and 10) of *Puccinia graminis avenae* have, at one time or another, been isolated from rusted oat material collected in various parts of the United States, only races 2 and 5 have played a significant part in the stem rust epidemics of this period. Race 1 was evidently of some consequence on certain occasions; but, on the whole, has been of trivial importance. Races 7 and 10, thus far, are of mere academic interest, so far as this country is concerned, but they may become more common in the future.

The restricted physiologic specialization of the oat stem rust fungus in the United States removes a serious impediment from the path of the plant breeder in his attempts to develop a desirable rust-resistant variety of oats. Adequate resistance to the dominant races 1, 2, and 5 is available in the varieties included in oat group 2, as listed in table 2. These varieties also are highly resistant to race 7. The varieties comprising oat group 1 are at least moderately resistant to the common races, as well as to race 10. To guard against the introduction or invasion of the more virulent races, it might be well to plan for the development of oat varieties with desirable agronomic characters that would resist their attack. Greenhouse experiments with seedling plants may facilitate and expedite the attainment of this goal.

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QUANTITATIVE DETERMINATION OF COPPER AND ESTIMATION OF OTHER TRACE ELEMENTS BY SPECTROGRAPHIC METHODS IN WIRE GRASSES FROM "SALT SICK" AND HEALTHY AREAS [1]

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INTRODUCTION

"Salt sick," a nutritional anemia, occurs among cattle, sheep, and swine, the feed of which is restricted to forage grown on certain types of sandy or peat soils. Earlier work of the Florida Agricultural Experiment Station demonstrated that the condition was caused by a deficiency of iron, or iron and copper, in the soils and forage (*2*).[3] Forage from salt sick areas was shown to be low in iron (*16*), and cattle grazing on these areas recovered when given an iron-copper supplement, as indicated by physical condition and hemoglobin content of whole blood (*15*). On certain soils, the affected animals responded erratically when iron was given as the sole supplement, but recoveries were quite uniform when a copper-iron supplement (1:50) was used.

A nutritional anemia having external and other symptoms similar to salt sick (*3*) has been reported as "bush sickness" or "skinnies" in New Zealand, "daising," "pining," "pine," and "vinquish" in southern Scotland, "nakuruitis" in Kenya Colony, East Africa, and "coasty disease" in King Island, Tasmania. An addition of cobalt to the ration was found to be helpful in certain parts of Australia and New Zealand (*1*, *21*). In Florida, iron and copper have been used successfully in correcting pure salt sick in field trials.

Cattlemen in Florida have long followed the practice of "burning the woods" (*4*) in order to remove old growth and give their animals the advantage of access to young vegetation in the late winter and early spring.

WORK OF OTHER INVESTIGATORS

The average iron content of wire grass, reported previously from this station (*16*), was 0.0177 percent in the dry matter of the grass (burned area) and 0.0168 percent (unburned area) from the deficient ranges, in contrast to 0.022 percent (burned) and 0.0193 percent (unburned) on healthy ranges. No analyses of grasses to determine their copper content have been reported from the Florida areas. The copper content of forage crops from other areas has been reported by a few investigators, as shown in table 1.

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[2] The authors are indebted to Drs. O. C. Bryan and R. B. Becker for collecting the grasses used in this study, and to Drs. R. B. Becker and W. M. Neal for determining the nutritional quality of the ranges.
[3] Reference is made by number (italic) to Literature Cited, p. 737.

TABLE 1.—*The copper content of forage crops reported by different investigators*

Forage crop	Method of analysis	Investigator	Copper per kilogram of dry matter
			Milligrams
Bluegrass	Xanthate	McHargue (*10*)	7.5
Kentucky bluegrass	Ferrocyanide	McHargue (*11*)	7.5
Alfalfa foliage			8.0
Red clover foliage			8.0
Kentucky bluegrass	Xanthate	McHargue (*12*)	14.0
Alfalfa hay:	Biazzo (modified)	Elvehjem and Hart (*5*)	9.1
First cutting (Wisconsin)			4.5
Second cutting (Wisconsin)			14.8
Colorado, sample 1			6.9
Colorado, sample 2			11.9
Bluegrass			8.3
Oat straw			3.8
Red clover hay			17.6
Rye straw			4.4
Soybean hay			8.2
Sweetclover hay			11.8
Timothy hay			2.2
Vetch hay			9.6
Wheat straw			2.1
Alfalfa hay	Xanthate	McHargue, Roy, and Pelphrey (*13*).	10.0
Alsike clover			6.0
Bluegrass hay			14.0
Orchard grass hay			5.0
Red clover hay			17.0
Redtop hay			4.0
Soybean hay			8.0
Sweetclover			9.0
Timothy hay			5.0
Wheat straw			3.0
Lecksucht area hay, average	Biazzo	Sjollema (*19*)	2.0–3.0
Lecksucht area hay, one case			3.0–5.0
Do			1.0
Normal hay, average			6.0–12.0
Normal hay, one case			5.0
Normal grass on pasture			20.0–30.0

METHODS OF INVESTIGATION

Samples of wire grass (*Aristida* sp.) were collected simultaneously with samples of soil (*3*) by plucking from areas on which cattle became salt sick, and on healthy areas from both burned and unburned ranges within a 2 months' period. The wire grass samples were analyzed spectrographically for quantitative differences in mineral content. The grass was dried at about 70° C. and stored in sealed glass jars. The samples selected for analyses were obtained from extreme salt sick, marginal,[4] and healthy areas.

All glass, platinum, and silica apparatus and equipment used were leached with hot 1 : 1 hydrochloric acid and rinsed several times with water obtained by triple-distillation through an acid-leached all-glass still. Spectrographic examination showed that this water contained less than one-half part per million of copper (*17*).

In earlier work (*16, footnote p. 251*) the senior author found that grinding wire grass in a Wiley mill increased its iron content by 0.0032 percent. To avoid contamination with metals, therefore, the grasses were broken by hand, placed in platinum or silica dishes, and dried at 100° C. After drying, the grasses were ashed for 24 to 48 hours in a muffle furnace at a temperature not exceeding 450°. The ash was then homogenized in an agate mortar and analyzed spectrographically.

[4] On marginal areas cattle do not develop salt sick, but affected cattle pastured on these areas do not recover.

QUANTITATIVE SPECTROGRAPHIC DETERMINATION OF COPPER

Many workers have reported quantitative spectrographic methods for the determination of copper in various materials. Gerlach and his coworkers (*7*, *8*, *18*) determined copper in biological materials with silver or cobalt as the internal standard; in this study cadmium was used.

To an accurately weighed portion of the ash of the sample a measured volume of a cadmium solution was added. This mixture was dried, carefully homogenized in an agate mortar, and small portions spectrographed on a Littrow spectrograph. Selected and tested purified graphite electrodes were used. In this connection, it should be noted that the best grade of graphite electrodes on the market frequently contains some copper, which may produce erratic results unless the electrodes are tested spectrographically prior to using, as was done in this study. Satisfactory and less expensive electrodes may be prepared by the purification methods of Standen and Kovach (*20*).

Great care was exercised at all times to avoid contamination. The solution of cadmium is known to contain a small proportion of copper, but the quantity thus added was negligible and was found not to affect the results.

Quintuplicate analyses of each sample were made. A nonrecording microphotometer was employed for measuring the ratio of the line densities. The probable error of the method is less than 10 percent of the mean.

RESULTS

The analyses for copper in the wire grasses from both healthy and salt sick areas that had been burned, or left unburned, are given in table 2. This table also presents the percentage of total ash on the dry-matter basis. The copper content of the wire grass samples from the salt sick areas ranged from 4.7 to 10 mg per kilogram of dry matter; those from the healthy areas ranged from 5.5 to 10.1 mg.

TABLE 2.—*Ash and copper content of wire grasses from salt sick and healthy (burned and unburned) areas as determined by spectrographic method*

Area, treatment, and sample no.	Ash (dry basis)	Copper per kilogram of dry matter [1]
Salt sick area, unburned:	*Percent*	*Milligrams*
223	2.6	6.1±0.6
243	3.1	7.6± .8
251	2.9	5.4± .5
261	2.6	5.3± .5
262	2.6	4.8± .5
263	2.7	4.7± .5
265	2.9	7.4± .7
275	2.6	4.8± .5
285	3.1	10.0±1.0
Average		6.2
Salt sick area, burned:		
237	2.5	6.6± .7
241	3.0	8.0± .8
258	3.3	9.3± .9
Average		8.0
Healthy area, burned, marginal: [2]	*Percent*	*Milligrams*
234	3.4	8.3± .8
245	2.8	6.3± .6
249	2.8	7.3± .7
292	2.8	5.5± .5
Average		6.8
Healthy area, unburned:		
230	3.7	9.6±1.0
232	3.9	7.5± .7
235	6.2	10.1±1.0
239	3.8	8.0± .8
270	3.4	8.6± .9
277	2.8	6.6± .7
283	3.0	5.9± .6
Average		8.0

[1] Mean of 5 determinations.

[2] This area not sufficiently supplied with available nutrients to be classed as strictly healthy (corrective), yet not so low as to be classed as salt sick.

DISCUSSION

A comparison of the copper content of samples of wire grasses collected from ranges on which cattle showed severe symptoms of salt sick, and those from ranges on which the animals were always healthy, failed to show any appreciable difference (table 2).

Neal and Becker (*16*), however, found that in the grasses from healthy areas the content of iron, calcium, magnesium, and phosphorus was higher than in the grasses from affected areas. An examination by Bryan and Becker (*3*) of the soil from both areas showed that the surface soils of the healthy ranges contained approximately 10 times as much iron, 5 times as much calcium and phosphorus, and twice as much copper as did those from the salt sick areas. These investigators report that "cattle will develop 'salt sick' on soils with 0.036 percent iron and 3.85 p. p. m. of copper, while they remain healthy upon soils with 0.42 percent of iron and 8 p. p. m. of copper."

The average values for copper in the grasses analyzed in the present work varied from 6.2 to 8 p. p. m. in salt sick and 6.8 to 8 p. p. m. in healthy areas, which indicates that the content of this element is not proportional in the vegetation and in the soil.

Since the grasses used in this work were obtained from extreme salt sick and healthy areas, the comparison of the copper content of these grasses indicates that copper deficiency per se is not the main etiological factor of salt sick although iron supplemented with copper was found to be effective in treating this condition in cattle. In the Netherlands, however, Sjollema (*19*) found an increase of about 5 p. p. m. in the copper content of hay from healthy areas as compared with hay from "Lecksucht" areas.

SPECTROGRAPHIC ESTIMATION OF OTHER TRACE ELEMENTS

Since no significant differences were found in the copper content of the wire grasses, it was decided to examine the ash samples for other trace elements. The procedure was designed to permit an estimation of the proportions of certain elements present in magnitudes detectable by spectrography on the original ash sample.

In this work no effort was made to increase the proportions of the elements in the samples by precipitation or any other chemical method. By concentration methods it probably would have been possible to detect the presence of some elements which are reported as "not detected;" but the fact that an element is found to be present in a very minute quantity is of less practical importance than a knowledge of its proportion in the sample. Accordingly, it seemed best to base this study on proportions of the elements detectable spectrographically in the ash of the sample.

A small proportion of the homogenized ash was volatilized in a 220-volt arc, a current of 9 to 10 amperes being used. Specially purified graphite electrodes were employed; repeated spectra of the graphite electrodes were made to insure a control of electrode impurities. In taking the spectrum of the sample, the arc was maintained until the sample was completely volatilized. Incomplete volatilization permits fractionation, which might involve a retention of the higher boiling elements in the residue and this vitiate the estimates of the amounts of the elements present.

A Littrow spectrograph with linear dispersion of about 30 inches between 2,250 A and 5,500 A was used. Two prisms were employed

with this instrument—a glass prism for lines of wave length greater than 3,800 A and a quartz prism for shorter wave lengths. To make use of the sensitiveness of the 2,138 A zinc line, a quartz Cornu type spectrograph with linear dispersion of about 9 inches between 2,100 A, and 8,000 A was employed for the zinc determinations.

The estimations were made by a modification of the comparison method used by Nitchie (*14*). Standard powders containing known percentages of the 24 elements included in the analysis were spectrographed in juxtaposition with spectrograms of the ash samples. The percentages of the elements present were then estimated by comparing visually the intensities of the spectral lines in the sample with corresponding lines in the standards. Duplicate determinations were made on each sample.

The data thus obtained are not intended as precision determinations, but are indicative of the "order of magnitude" of the proportions of the elements present. To avoid misunderstanding as to precision, and also to retain a legitimate basis for comparison, the data are presented in "range" form. For example, 1–10 recorded in table 3 should be read: The amount of the element in the sample lies between 1 and 10 mg per kilogram of dry matter.

The approximate sensitivity of the method is: For chromium, cobalt, nickel, and silver, between 0.0001 and 0.001 percent; for aluminum, barium, lead, manganese, molybdenum, strontium, tin, titanium, vanadium, yttrium, and zinc, between 0.001 and 0.01 percent; for antimony, boron, bismuth, cadmium, lanthanum, thallium, and zirconium, between 0.01 and 0.1 percent; and for arsenic and lithium between 0.1 and 1 percent.

The term "trace" signifies the smallest detectable proportion of the element in the ash. This value converted to the dry-matter basis is almost negligible, but this does not alter the fact that the element was detected in the ash.

RESULTS

The spectrographic estimations of the trace elements in the same wire grass samples that were used for the copper determinations are shown in table 3. Aluminum, barium, boron, lead, manganese, strontium, titanium, and zinc were detected in all the ash samples, in addition to copper.

Of the grasses from the unburned salt sick area, chromium and silver were detected in traces in eight of the nine ash samples, and molybdenum in five. All the elements mentioned above except molybdenum were present in grasses from the burned salt sick and from the burned marginal areas. Molybdenum was detected in two of the three ash samples from the burned affected area, and in two of the four samples from the burned marginal area.

Chromium was detected in all the ash samples from the unburned healthy areas, in addition to the 10 elements present in all the grass samples from the burned areas. Molybdenum and nickel were detected in three of the seven ash samples in this group.

Antimony, arsenic, beryllium, bismuth, cadmium, cobalt, lanthanum, thallium, tin, vanadium, yttrium, and zirconium were not detected in any of the ash samples from these areas. Nickel was detected only in the grasses from the healthy unburned area, and in one sample from the unburned salt sick area.

TABLE 3.—*Spectrographic estimation of trace elements in wire grasses from salt sick and healthy areas expressed in milligrams per kilogram of dry matter* [1]

[Analyses made on ash, but for convenience converted to dry-matter basis. Duplicate analyses made]

Sample no.	Aluminum	Barium	Boron	Chromium	Lead	Manganese	Molybdenum	Nickel	Silver	Strontium	Titanium	Zinc
Salt sick area, unburned:	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*
223	20–80	1–10	10–30	Tr	1–10	20–80	ND	ND	ND	<0.1	1–10	2–20
243	2–20	<0.1	2–20	Tr	1–10	10–30	Tr	ND	<0.1	<.1	0.1–1	30–100
251	10–30	.1–1	2–20	Tr	0.1–1	10–30	ND	ND	Tr	<.1	.1–1	10–30
261	20–80	1–10	2–20	Tr	.1–1	20–80	ND	ND	Tr	<.1	<0.1	20–80
262	10–30	1–10	2–20	Tr	.1–1	2–20	<0.1	ND	Tr	<.1	.1–1	10–30
263	10–30	<.1	10–30	ND	.1–1	2–20	<.1	ND	Tr	<.1	<.1	20–80
265	10–30	.1–1	2–20	Tr	1–10	1–10	Tr	Tr	<.1	<.1	<.1	20–80
275	2–20	.1–1	1–10	Tr	.1–1	10–30	Tr	ND	Tr	<.1	<.1	20–80
285	10–30	.1–1	2–20	Tr	.1–1	30–100	ND	ND	Tr	<.1	<.1	30–100
Salt sick area, burned:												
237	10–30	1–10	2–20	Tr	.1–1	20–80	0.1–1	ND	Tr	<.1	.1–1	10–30
241	20–80	1–10	2–20	Tr	.1–1	30–100	<.1	ND	Tr	<.1	1–10	10–30
258	1–10	<.1	2–20	Tr	.1–1	1–10	ND	ND	Tr	<.1	<.1	30–100
Healthy area, burned, marginal:												
234	30–100	.1–1	2–20	<0.1	1–10	10–30	ND	ND	Tr	<.1	1–10	1–10
245	20–80	1–10	2–20	<.1	.1–1	>60	.1–1	ND	Tr	<.1	.1–1	20–80
249	20–80	1–10	2–20	<.1	.1–1	10–30	.1–1	ND	Tr	<.1	.1–1	10–30
292	10–30	.1–1	2–20	Tr	.1–1	20–80	ND	ND	Tr	<.1	<.1	10–30
Healthy area, unburned:												
230	15–50	1–10	2–20	Tr	1–10	15–50	ND	ND	Tr	<.1	.1–1	30–100
232	30–100	1–10	2–20	Tr	1–10	10–30	ND	Tr	Tr	.1–1	.1–1	1–10
235	>60	20–80	10–30	<.1	1–10	>60	ND	0.1–1	Tr	<.1	2–20	1–10
239	30–100	2–20	2–20	Tr	.1–1	15–50	1–10	ND	Tr	<.1	1–10	15–50
270	15–40	.1–1	10–30	Tr	1–10	1–10	ND	ND	Tr	<.1	1–10	30–100
277	10–30	1–10	2–20	Tr	.1–1	20–80	Tr	Tr	<.1	<.1	<.1	20–80
283	2–20	10–30	2–20	Tr	<.1	10–30	1–10	ND	Tr	<.1	Tr	10–30

[1] ND denotes not detected. Tr denotes trace (see text for discussion). Antimony, arsenic, beryllium, bismuth, cadmium, cobalt, lanthanum, thallium, tin, vanadium, yttrium, and zirconium were not detected in any of the ash samples.

DISCUSSION

A comparison of the values for the trace elements in the wire grasses from healthy and affected areas did not show any appreciable differences.

Certain elements were detected in all the ash samples; hence, the results for these elements are at least consistent. Other elements were detected irregularly, which indicates that the ash may have been contaminated.

A high-titanium content in green parts of certain plants has been reported by Geilmann (*6*) and Headden (*9*), and unpublished work at this laboratory has confirmed this finding. In the present study, titanium was detected in all the ash samples, but the proportions were low as compared with those reported by Geilmann and Headden.

Recently, workers at this station have found that cobalt is a limiting factor in one type of salt sick known specifically as "hill sick." The estimation of trace elements failed to show the presence of cobalt in any of the grass samples (table 3), yet this element is known to be beneficial to animals on certain areas. It should be pointed out that some of the elements reported in this study as not detected may have been present, but in amounts too small to be determined by the method used.

The results obtained from this study suggest that salt sick may be the result of a combination of conditions which give rise to similar external symptoms. The ratio of the various elements involved may

be as important as the absolute amounts. Possibly salt sick may occur on grasses in which the iron content is high and the copper content low, or vice versa; or the ratio of iron or copper to some other element or elements may be involved.

Another factor that should be considered is the mineral content of the drinking water. In some water holes where an iron-bearing clay forms the substrate, the water is known to have a high content of iron and probably of other elements.

SUMMARY

A comparison of the results of spectrographic determination of the copper content of wire grasses from "salt sick", marginal, and healthy areas failed to disclose any significant differences.

The spectrographic estimation of other trace elements also showed no significant differences.

The following elements were detected in all the wire grass samples from the different areas: Aluminum, barium, boron, copper, lead, manganese, strontium, titanium, and zinc. The following elements were not detected in any of the samples: Antimony, arsenic, beryllium, bismuth, cadmium, cobalt, lanthanum, thallium, tin, vanadium, yttrium, and zirconium. Chromium, molybdenum, silver, and nickel were detected in the samples, but not consistently.

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FACTORS INFLUENCING THE DEVELOPMENT OF SOGGY BREAK-DOWN IN APPLES[1]

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INTRODUCTION

The influence of storage temperature on soggy break-down[2] in certain apple varieties is generally recognized, but the relation of other etiologic factors to this disease is not so clearly understood. For example, it has been assumed that delayed storage is essential to the development of soggy break-down, and that late picking favors its occurrence. The effect of these and other causal agencies has not been demonstrated conclusively. Among other factors which have been considered as favorable or essential to the development of soggy break-down are inadequate ventilation of containers and storage rooms, a prolonged storage period, temperatures lower than 32° F., variations in fertilizer treatments, and climatic conditions. The effect of these and certain other factors on soggy break-down is considered in this paper.

The importance of storage temperature in the control of soggy breakdown has been emphasized in earlier publications (*11*, *15*, *17*).[3] This paper presents further evidence to show the relation of storage temperature to the disease in the varieties that were previously listed as susceptible and in other varieties for which little or no information is on record. The relative importance of other storage and prestorage factors as judged by the results of more than 11 years' work is indicated.

REVIEW OF LITERATURE

Investigators have emphasized the importance of immediate storage in the control of the soft scald type of soggy break-down in the Jonathan apple (*1*, *3*, *6*, *18*, *19*). In an earlier paper (*13*) the writers indicated that delaying storage for 1 week, as compared with immediate storage or storage after 2 and 3 weeks' delay, usually increased susceptibility in this variety. It should be noted, however, that immediate storage did not invariably prevent the disease, and that delays of 2 and 3 weeks gave best control. On the other hand, delaying the storage of Grimes Golden and Golden Delicious has usually resulted in greater susceptibility to soggy break-down (*10*, *15*). Harding found that cold-storing Grimes Golden while it is in an active state of respiration produces a high degree of susceptibility (*4*), and that it reaches its highest respiratory activity between 3 and 5 weeks at 50° F.

Harley and Fisher (*6*) concluded that a high concentration of carbon dioxide in Jonathan apple tissues is not a contributory cause of soggy break-down; Brooks and Harley (*2*) found that short exposures to

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[2] The two types of low-temperature disease designated in the literature as "soggy break-down" and "soft scald" are considered by the writers to be the same, and in this paper will be included under the term "soggy break-down." The reasons for this classification have been given (*11*, *17*).

[3] Reference is made by number (italic) to Literature Cited, p. 762.

this gas and high temperature reduced the tendency toward the disease. Gerhardt and Ezell (*3*), working with Jonathan at Wanatchee, Wash., found that immediate storage at 32° F., when preceded by a 24-hour exposure to carbon dioxide, gave approximately as good control as ordinary storage at 36°. With delayed storage, however, carbon dioxide exposures gave less consistent results.

RELATION OF STORAGE TEMPERATURE TO SOGGY BREAK-DOWN

Although results of storage experiments of a rather comprehensive nature, showing that temperature is of primary importance in the control of soggy break-down in Grimes Golden, have been published (*11, 15, 17*), information concerning the effects of storage temperature on other varieties is limited. Some results for Jonathan, Wealthy, and Golden Delicious are on record (*9, 10, 14, 15*), but so far as the writers can determine, no results have been published for Northwestern Greening and Winter Banana.

STUDIES WITH JONATHAN

TABLE 1.—*Percentage of soggy break-down occurring in Jonathan apples during storage at different temperatures for three and for five seasons*

RESULTS DURING 3 SEASONS [1]

Temperature (° F.)	Delay before storing	Break-down in— [2]		
		1924–25	1926–27	1928–29
	Weeks	*Percent*	*Percent*	*Percent*
30	0	42.3	14.1	8.2
	1	18.9	6.9	67.6
	2	10.3	2.9	21.7
	3	0	1.0	15.2
32	0	6.9	0	1.8
	1	.6	0	19.1
	2	.6	0	16.2
	3	0	0	.7
34	0	0	0	0
	1	0	0	1.9
	2	0	0	5.1
	3	0	0	0
36	0	0	0	0
	1	0	0	.8
	2	0	0	.9
	3	0	0	0
Common storage	0	0	0	0
	1	0	0	0
	2	0	0	0
	3	0	0	0

RESULTS DURING 5 SEASONS [3]

Temperature (° F.)	Delay before storing	Break-down in— [4]				
		1929–30	1930–31	1931–32	1932–33	1933–34
	Days	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
30	0	76.2	8.7	0.0	11.2	0.7
	10	28.2	0	12.2	3.5	16.0
	20	1.4	0	0	0	0
36	0	0	0	0	0	0
	10	0	0	0	0	0
	20	0	0	0	0	0

[1] Fruit examined the latter part of January.

[2] Picking dates: 1924, October 7; 1926 and 1928, October 2. Source of Fruit: 1924, State orchard, western Iowa; 1926 and 1928, Apple Grove orchards, central Iowa. Number of boxes per test: 1 to 4. The same investigation was carried out in the seasons of 1925 and 1927. No soggy break-down occurred in these 2 years.

[3] Fruit examined in February 1929–30 and 1931–32 and in March 1930–31, 1932–33, and 1933–34.

[4] Picking dates: 1929 and 1931, October 2; 1930, Oct. 6; 1932, September 30. Source of fruit: 1929, 1930, and 1931, Apple Grove orchards, central Iowa; 1932 and 1933, Worth orchard, western Iowa. Number of boxes per test: 1 to 4. In 1934 there was no crop because of severe drought.

The effect of various storage temperatures on the development of soggy break-down in Jonathan is shown in table 1. These results show that break-down developed more severely at 30° F. than at 32°, 34°, or 36°, that it was a factor at 32°, and that it was not entirely absent at 34°. At 36° only traces of the disease appeared, and in common storage there was none. Experiments similar to those reported for three seasons in table 1 were also carried out in the seasons of 1925–26 and 1927–28, but no soggy break-down occurred. In

FIGURE 1.—Sections of Jonathan apples severely affected with the soft scald or surface type of soggy break-down. In most of the specimens shown the disease extends deeper into the cortex of the apple than is usual. (See fig. 8 for typical external appearance of soggy break-down in Jonathan.)

earlier work (*13*) the writers found extensive development of soggy break-down at 32° in each of three other seasons. The difference in the susceptibility of Jonathan in various years is thus very marked. The tendency to break-down in different lots of fruit given various delayed treatments also varied in different years. This factor is considered later.

The results of storing Jonathan at the two temperatures 30° and 36° F. for five additional storage seasons are shown in table 1. These studies also show complete control of the disease at 36°. Variation in the susceptibility of Jonathan in different years was again apparent, and dissimilar break-down tendencies between lots of fruit given different delayed treatments were also marked. The results as a whole show that at a temperature of 36° soggy break-down (fig. 1) was

completely eliminated in each of seven seasons and that it was kept under 1 percent in each of eight.

STUDIES WITH NORTHWESTERN GREENING

Storage experiments in which boxes of Extra Fancy Northwestern Greening apples were stored at various temperatures for each of two seasons are shown in table 2. In both seasons the fruit tested came from trees approximately 25 years old, and was picked at the peak of the picking season. The number of fruits per box ranged from 125 to 72, but the majority of the boxes contained between 113 and 88. No color distinction between green and yellow fruit was made. The study also includes 2 years of observations of this variety under common storage. Delay before storing took place in an open packing shed.

TABLE 2.—*Percentage of soggy break-down occurring in Extra Fancy Northwestern Greening apples* [1] *at different temperatures* [2]

Storage temperature (° F.)	Delay before storing	Slight break-down		Severe break-down	
		1924–25	1927–28	1924–25	1927–28
	Days	*Percent*	*Percent*	*Percent*	*Percent*
30	2	12.5	12.7	66.4	8.7
	7	12.5	16.8	17.3	8.4
	14	1.9	0	0	0
	21		13.4		41.3
32	2	12.2	28.4	41.4	43.2
	7	13.6	21.8	29.6	0
	14	0	2.6	0	.9
	21		14.9		4.4
34	2	1.5	42.7	1.0	17.3
	7	4.8	15.3	0	4.2
	14	0	0	0	0
	21		2.4		2.4
36	[3] 2	6.8	56.3	2.6	11.3
	7	1.6	5.1	.8	0
	14	0	1.7	0	0
	21		0		0
40	1	4.4		2.7	
	7	3.8		1.9	
	14	1.0		1.0	
29–60 [4] (common storage)	1	0		0	
	7	0		0	
	14	0		0	

[1] Picking date, October 13 in both seasons. Source of fruit: 1924, State orchard, western Iowa; 1927, Apple Grove orchards, central Iowa.
[2] Fruit examined February 18 for 1924–25 and March 3 for 1927–28.
[3] 1-day delay in 1924–25.
[4] Minimum and maximum temperatures for various months that fruit was in common storage: October, 44°–60° F.; November, 32°–54°; December, 32°–39°; January, 29°–34°.

The severity of the disease is expressed as slight break-down, which includes apples with an approximate total affected area not more than one-half inch in diameter; and severe break-down, which includes all specimens more severely affected. Severely affected apples frequently have 50 to 75 percent or more of their areas involved, and are always worthless. Slightly affected apples usually have some culinary value. At 36° F. Northwestern Greening shows a rather high proportion of slightly affected fruit, while at the lower temperatures the proportion of severely affected fruits is greater.

Severe break-down was usually most prevalent at the two lowest temperatures, 30° and 32° F. (fig. 2 and table 2). The results were similar for the fruit recorded under slight break-down in 1924, but for the 1927 fruit they were somewhat less consistent. At 40° the percentage of break-down was under 5 percent, and none occurred in common storage. The tendency for the fruit to decrease sharply in susceptibility with delayed storage is conspicuous in this variety; fruit given 2 and 3 weeks' delayed treatment was practically immune to soggy break-down.

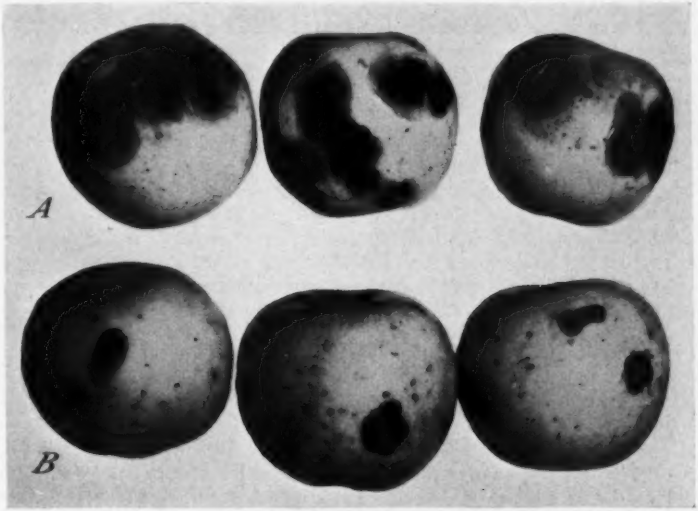

FIGURE 2.—Soggy break-down in Northwestern Greening: *A*, Severe soggy break-down occurring at 30° and 32° F.; *B*, slight soggy break-down in typical specimens stored at 36°. Like Jonathan, Northwestern Greening does not exhibit the internal type of soggy break-down.

Further evidence that storage temperature is a practical method of curtailing soggy break-down in Northwestern Greening is presented in table 3. The fruit used in this study was similar to that recorded in table 2 and received the same prestorage and storage treatment. Data for six seasons are presented on storage lots which were stored in most instances on the day of picking, or 1 or 2 days later. Except in one instance, break-down was most severe in fruit stored at 30° F. Fruit at 32° consistently yielded higher percentages than that at 34°, and the latter usually higher than fruit at 36°. No break-down occurred in common storage. The rather large percentage of break-down at 36° is indicative of the high degree of susceptibility of Northwestern Greening. This apple is regarded as the most susceptible variety thus far studied at Ames. The smaller amount of break-down noted in the 1925 data is probably the result of the delayed storage treatment of 11 days.

TABLE 3.—*Percentage of soggy break-down [1] occurring in Extra Fancy Northwestern Greening apples [2] during storage at different temperatures, 1924–29*

Year	Picking date	Delay before storing	Date examined	Break-down at storage temperature [3] of—			
				30° F.	32° F.	34° F.	36° F.
		Days		*Percent*	*Percent*	*Percent*	*Percent*
1924	Oct. 13	2	Feb. 18	66.4	41.4	1.0	2.6
1925	Oct. 9	11	Feb. 20	9.0	2.2	0	0
1926	Oct. 8	1	Jan. 13	41.6	27.3	11.2	5.6
1927	Oct. 13	2	Mar. 3	8.7	43.9	17.6	11.8
1928	Oct. 20	0	Mar. 22	46.3	28.8	10.4	8.7
1929	Oct. 8	0	Jan. 23	62.0	----------	----------	18.7

[1] Soggy break-down given as severe break-down; slight break-down excluded.
[2] The 1924 lot of fruit was from the State orchard in western Iowa; that for 1925–29 all came from Apple Grove orchards in central Iowa. In all cases fruit was from trees approximately 25 years old.
[3] No break-down occurred in common storage.

STUDIES WITH WEALTHY

In previous publications (*14*, *15*) the writers have reported the occurrence of soggy break-down in the Wealthy variety. A distinction was then made between soft scald and the internal type of soggy break-down; so that the data do not give a true picture of the severity of the latter disease in this variety. The internal type of soggy break-down was not recognized as such until some years later; and the earlier data (*14*, *15*) therefore include only the soft-scald type (fig. 3).

In the studies for the three seasons reported in table 4, the apples were picked in prime condition, were of good color, and of Extra Fancy grade. They were wrapped in oiled-paper wrappers and packed in boxes. The fruit in all cases was selected from old trees.

There was no soggy break-down of fruit in common storage, and when the disease occurred at 36° F., in most instances the percentage was low. Storage at 34° did not control the break-down satisfactorily, particularly when storage was delayed, and storage at 30° and 32° in most cases was even less favorable. In general the data indicate that 36° is the most desirable temperature for the prevention of soggy break-down, and that delayed storage up to a certain point increases the susceptibility of the fruit. Beyond this point, according to the 1928 data, delayed storage appears to increase resistance.

TABLE 4.—*Percentage of soggy break-down occurring in Wealthy apples during storage at different temperatures* [1]

Season	Picking date	Delay before storing	Break-down at storage temperatures [2] of—			
			30° F.	32° F.	34° F.	36° F.
		Days	*Percent*	*Percent*	*Percent*	*Percent*
1924–25	Sept. 17	1		0.0		
		7		32.8		
		14		38.4		
1926–27	Sept. 22	2	15.2	5.3	2.5	4.7
		9	22.0	12.3	6.0	1.2
1928–29	Sept. 5	2	1.7	2.3	3.0	0
		10	85.7	85.9	49.2	28.3
		14	79.6	89.8	44.4	11.3
		21	36.6	16.1	14.6	0

[1] Fruit examined in January in each season. Source of fruit: 1924 and 1926, northern Iowa; 1928, central Iowa.
[2] No break-down occurred in common storage.

STUDIES WITH WINTER BANANA

Attention was first called to the occurrence of soggy break-down in Winter Banana apples in 1933, when the writers observed a trace of the disease in one box of apples stored at 31° F. Results of storage tests for this variety are now available for three seasons (table 5). The apples came from northeastern Iowa; those tested in 1934 and 1935 were from trees 8 to 10 years old and those tested in 1936 were from trees approximately 25 years old, growing in a sandy loam soil. The fruit, of extra fancy grade, was packed and wrapped in oiled paper in 1934 and 1936, but was left unwrapped in 1935. The apples in the 1934 and 1936 seasons were picked on October 5 and in the 1935

FIGURE 3.—Soggy break-down in Wealthy apples: *A*, Surface type resembling, in external appearance, the disease as it occurs on Jonathan and Northwestern Greening. Sections of such specimens frequently reveal also the internal type. *B*, internal type in specimens exhibiting no external symptoms of the disease, though similar specimens frequently have both types of soggy break-down.

season on October 7. In 1935 and 1936 the fruit was considered normal in development; in 1934 it was slightly more mature than in the other two seasons. This might have been occasioned by extreme warm weather in the 1934 season.

It will be noted that the earliest stored fruit of the first season was most susceptible, and that there was a gradual decrease in the susceptibility of the apples given the 5 and 9 days' delay. With and following the 13-day delay there was usually marked decrease in susceptibility. The results for the second year differed from those of the first in that immediate storage and the shorter delays resulted in limited amounts of soggy break-down, while longer delays increased susceptibility. However, the tendency for the fruit given the longer delays (15 and 18 days) to increase in resistance, although not so marked, is apparent in the 1935 results, as it was in 1934. The data for storage at 36° are not complete, but the results indicate that Winter Banana apples are resistant to soggy break-down at this temperature.

TABLE 5.—*Percentage of soggy break-down occurring in Winter Banana apples during storage at different temperatures* [1]

Season	Apples stored at—		Delay at 50° F. before storing	Break-down in storage at 31° F.[2]	Season	Apples stored at—		Delay at 50° F. before storing	Break-down in storage at 31° F.[2]
	31° F.	36° F.				31° F.	36° F.		
	Number	*Number*	*Days*	*Percent*		*Number*	*Number*	*Days*	*Percent*
1934–35	113	125	1	73.5	1936–37	150	.....	0	1.4
	125	.....	5	60.2		163	.....	3	1.2
	290	.....	9	51.4		125	.....	6	7.2
	301	.....	13	16.6		163	.....	9	12.0
	301	.....	17	13.6		150	.....	12	5.4
	163	.....	21	4.3		138	.....	15	23.6
	150	.....	25	19.3		150	.....	18	29.5
1935–36	96	113	0	0		150	.....	21	55.0
	96	113	3	3.1		163	.....	24	16.6
	102	113	6	11.8		150	.....	27	3.3
	110	.....	9	68.2		163	.....	35	1.3
	109	.....	12	72.4		150	.....	42	0
	107	.....	15	57.8		150	.....	49	0
	102	.....	18	34.3					

[1] Fruit examined Feb. 15, 1935, Feb. 6, 1936, and Feb. 10, 1937.

[2] No break-down occurred in cold storage at 36° F., though only 1 test after 1 day delay was made in 1934–35 and 3 tests, after 0, 3, and 6 days' delay, respectively, were made in 1935–36. 1 box stored at 50° continuously in 1936–37 showed no break-down.

The results obtained in the third season confirm those of the second in that lowest break-down occurred with the shorter periods of delay, and susceptibility to break-down increased as the delayed storage period was increased to a certain degree. Highest susceptibility occurred with 21 days' delay in the 1936–37 season, and with 12 days' delay in 1935–36. The fruit of the first season was somewhat overmature and this probably accounts for the earlier stored samples of that year being more susceptible to soggy break-down.

Specimens of Winter Banana exhibiting the soft scald type of soggy break-down very frequently exhibit the internal type of break-down also, and both types occur concurrently in the same storage lots (*16*). These observations provide further evidence that the two types of soggy break-down are identical (fig. 4).

STUDIES WITH GOLDEN DELICIOUS

Golden Delicious is one of the most susceptible varieties and occasionally develops break-down at 34° and 36° F. Some results of experiments with this apple showing that a temperature of 36° controls soggy break-down have been published (*10*). It is sufficient to call attention to the fact that in these studies soggy break-down occurred severely at 30° and 32° F.; that 34° was unsatisfactory when storage was delayed 12 and 14 days; and that at 36° and in common storage the disease did not appear even though storage was delayed 3 weeks in some instances.

Further data for three seasons for fruits kept at a temperature of 31° F. likewise show the susceptibility of this variety to low-temperature break-down (table 7). Sufficient numbers of check boxes were kept at a temperature of 36° each year to confirm the resistance of Golden Delicious at the higher temperature.

RELATION OF DELAYED STORAGE TO SOGGY BREAK-DOWN

The effect of relatively short delays before storing and of prolonged periods of delay on the susceptibility of apples to soggy break-down varies with different varieties as well as with different seasons. In this discussion, then, consideration is given (1) to the effect of relatively

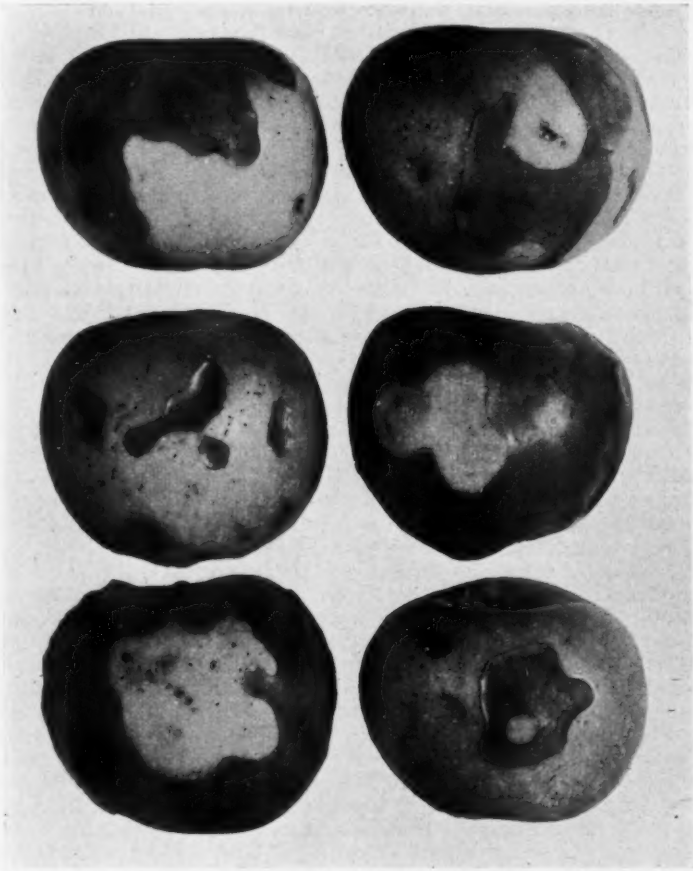

FIGURE 4.—Soggy break-down of Winter Banana apples. These specimens, although showing the typical surface type of soggy break-down, probably would also exhibit the internal type if sectioned.

short delays, and (2) to the effect of protracted delays on the subsequent development of soggy break-down in cold storage. The data presented are for typical seasons when the disease was prevalent in the varieties studied.

INFLUENCE OF RELATIVELY SHORT DELAYS

Unavoidable delays of a few days to several weeks sometimes occur in carrying out large-scale harvest operations. When this happens, the results may be expected to be considerably different from what

they would have been had the fruit been stored within 24 hours after picking.[4] Particularly is this true if the storage temperature is as low as 31° or 32° F.

From the results of the storage investigations at Ames, the writers have found it difficult to state definitely whether immediate storage or short delays result in greater susceptibility in Jonathan apples. In some instances immediate storage has made Jonathan more susceptible; in others delayed fruit has been more susceptible.

That delaying the storage of Jonathan may not necessarily increase susceptibility but, on the contrary, may decrease it, is indicated by the results shown in figure 5, *A*. Data are presented for four seasons, and these may be considered to give about the average picture over a period of years. In two seasons short delays of approximately a week to 10 days increased the tendency to break-down; in two other seasons a delay of 1 week as compared to immediate treatment, decreased susceptibility. With one exception, 2 to 3 weeks' delay made Jonathan more resistant, or about as resistant as immediate treatment. These observations show that susceptibility to low-temperature breakdown in Jonathan may be either positively or negatively associated with short delays before storage, according to the particular season in which the fruit is grown. Further evidence supporting this statement is given in tables 1 and 2.

That delayed treatment may definitely make apples more resistant to soggy break-down is indicated by results depicted in figure 5, *B*. The results for Northwestern Greening are typical for this variety (table 3). Results for the one season with Winter Banana, though similar to those for Northwestern Greening, are the reverse of those obtained in 2 other years (table 5); so that no definite statement as to the performance of this variety can yet be given. It may, like Jonathan, give on result one season and the opposite result another.

The usual or expected results of delaying storage of Golden Delicious and Grimes Golden apples are shown in figure 5, *C*. For these two varieties immediate storage, or a very short delay of 2 to 3 days, usually gives better results than longer delay. The two seasons' results for these varieties exemplify the general rule for the occurrence of soggy break-down. However, exceptions to this rule for Grimes Golden and possibly Golden Delicious may be expected. For example, data for 1924 (*15*) showed that Grimes Golden given only 24 hours' delay became susceptible to break-down, and data for another season showed that when grown during the very warm summer of 1934 under heavy irrigation it became very susceptible under immediate treatment (table 6). Regardless of exceptions, however, it is reasonably certain that delays of 1 to 3 weeks in the storing of Grimes Golden and Golden Delicious will result in higher susceptibility to soggy break-down than will immediate storage.

INFLUENCE OF PROLONGED DELAYS

As in the case of short delays, the effect of prolonging the period of delayed storage on the subsequent behavior of apples in storage varies considerably between varieties and seasons. In studying this problem, testing and observation over a period of years with susceptible varieties was necessary.

[4] The writers have data which indicate that a difference of 24 hours in the storing of Jonathan may result in marked differences in susceptibility.

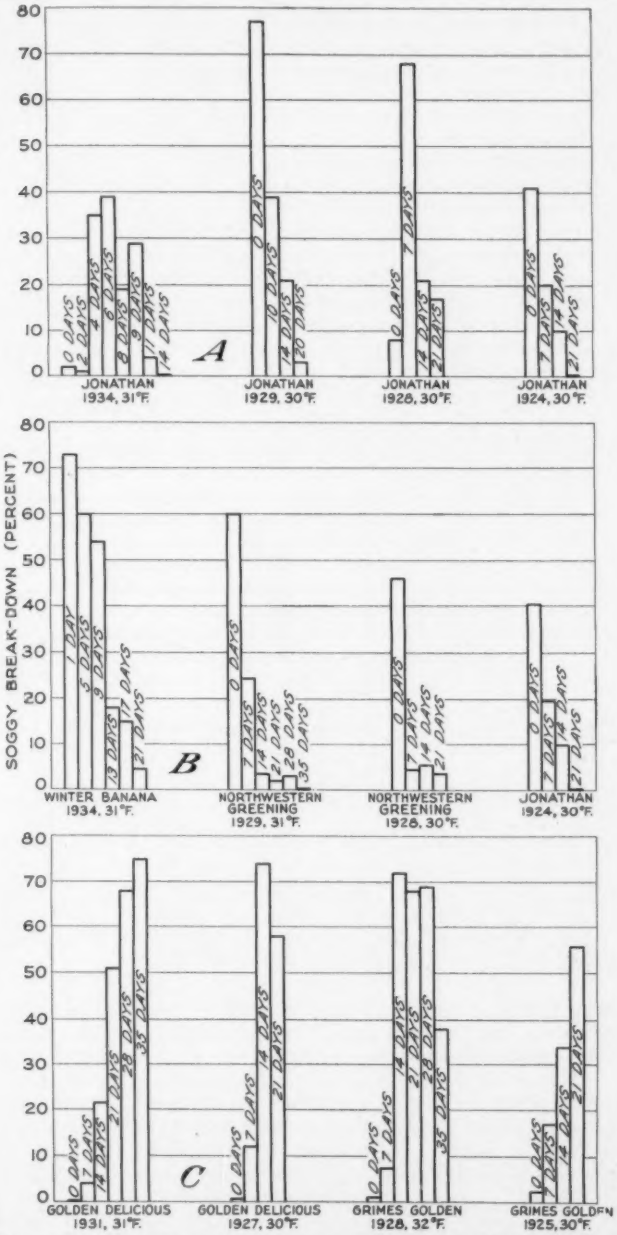

FIGURE 5.—Percentage of soggy break-down in apples of different varieties withheld from storage for various lengths of time and then stored at the temperatures shown. *A*, fruit held at 50° F. before storage, except in 1924 when it was held at outdoor temperatures; *B*, fruit held at 50° in 1929 and 1934 and at outdoor temperatures in 1924 and 1928; *C*, fruit held at 50° in 1928 and 1931 and at temperatures prevailing in an open packing house in 1925 and 1927.

Experimental studies since 1924 have shown that keeping Jonathan at ordinary outside temperatures 3 to 4 weeks or longer before cold storing makes it practically immune to soggy break-down.[5]

Results with Grimes Golden

The effect of protracted periods of delay on the development of soggy break-down in Grimes Golden for each of 5 years is recorded in table 6. After 6 weeks of delayed treatment in 1928 in each of three lots stored at different temperatures, there may be noted a definite breaking point in the susceptibility of the stored fruit. Six weeks' delay resulted in similar reductions in the susceptibility of the fruit in 1931 and 1934, while 5 and 7 weeks' delay yielded similar results in 1929 and 1930. In nearly all instances, therefore, protracted delays reduced the tendency to soggy break-down after definite periods of time. Periods of delay greater than 5 to 7 weeks did not increase susceptibility to this type of break-down.

TABLE 6.—*Effect of length of period of delay before storage upon the percentage of soggy break-down occurring in Grimes Golden apples* [1] *during storage at different temperatures in five seasons*

Lot No.	Delay at 50° F. before storing	Break-down in season and at temperature indicated								
		1928–29			1929–30		1930–31		1931–32	1934–35
		30° F.	32° F.	36° F.	30° F.	36° F.	31° F.	36° F.	31° F.	31° F.
	Weeks	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	0	3.8	0.4	0.4	4.7	0.0	0.0	0.0	0.0	68.5
2	1	.8	6.5	.6	------	------	1.5	0	.6	97.0
3	[2] 2	67.8	73.5	4.0	24.6	0	5.6	0	1.7	63.4
4	3	60.3	67.9	8.7	41.0	0	9.3	0	2.4	43.7
5	4	63.4	69.3	21.4	7.4	0	13.4	0	2.2	44.8
6	5	68.5	38.0	8.1	2.3	0	5.6	0	2.5	36.1
7	6	.7	3.5	0	0	0	16.9	0	1.2	7.5
8	7	.8	0	0	0	0	2.2	0	0	6.1
9	8	.6	0	0	0	0	1.5	0	0	.6
10	9	0	0	0	------	------	------	------	0	0
11	10	0	0	0	------	------	------	------	------	0
12	11	0	0	0	------	------	------	------	------	0
13	12	0	0	0	------	------	------	------	------	------

[1] Source of fruit: 1928 and 1929, old trees, Apple Grove orchards, central Iowa; 1930 and 1931, young trees, Highland fruit farm, western Iowa; 1934, young trees, Schurk orchard, southeastern Iowa. Growing conditions: 1928 and 1929, temperature moderate, rainfall abundant; 1930 and 1931, temperature excessively high, soil moisture unusually low; 1934, temperature very high, orchard under irrigation. Picking dates: Sept. 25, 1928; Sept. 26, 1929; Sept. 16, 1930; Sept. 22, 1931; Sept. 25, 1934. Fruit examined: Jan. 24, 1928; Jan. 29, 1929; Apr. 1, 1930; Feb. 19, 1931; Feb. 15, 1934. 1 box in each test.

[2] 10 days' delay in 1929–30.

In table 6 it may be noted that in 1934 the marked predisposition to soggy break-down in promptly stored Grimes Golden was accompanied by low break-down tendencies under prolonged delayed treatments. This fruit from a heavily irrigated orchard in southern Iowa was grown during the warmest summer on record and was picked when very mature, yet it improved in resistance with the longer delayed treatments. It appears, therefore, that Grimes Golden becomes markedly less susceptible to soggy break-down when given a 6-week

[5] It seems advisable to point out that the practice of delaying the storage of fruit either for short periods or for long periods is not recommended in any instance as a measure for the prevention of soggy break-down. The experiments on delayed storage have been conducted mainly for the purpose of acquiring information pertaining to the nature of this disease. Delaying storage of apples may make them more resistant to soggy break-down, but it may also make them more susceptible to other diseases, such as apple scald, Jonathan spot, bitter pit, mealy break-down, or to loss in flavor, mealiness, etc.

prestorage treatment at 50° F., and practically immune with still longer, similar prestorage treatments.

Harding's respiration studies (*5*) are of particular significance in view of the foregoing results. He found that high respiratory activity (in the 1928–29 and 1929–30 fruit, table 6) was correlated with high susceptibility to soggy break-down. In other words, both the respiration rate and the liability to soggy break-down were noticeably lowered after approximately 6 weeks of storage at 50° F.

Results with Golden Delicious

In experiments with Golden Delicious, comparable to those carried on with Grimes Golden and Jonathan, the results were similar but less conclusive. Data for 3 years of observations are reported in table 7. Relatively long delays of 8 to 12 weeks at 50° F. somewhat decreased the severity of soggy break-down in the 1931 and 1934 fruit. After 9 and 10 weeks of delayed treatment in 1931 there was a distinct lowering of susceptibility, the percentages being 31.5 and 44.6 respectively, as compared to 57.3 for the 8 weeks' treatment. There was a gradual decrease in susceptibility beginning with the 7 weeks' treatment, but the differences were less marked than with Grimes Golden. Decreases in susceptibility to severe break-down in the 1934 fruit are similar to those in the 1931 fruit. As the tendency to severe break-down decreased in the 1934–35 fruit, the tendency to slight break-down increased.

Table 7.—*Effect of length of period of delay before storage upon the percentage of soggy break-down occurring in Golden Delicious apples* [1] *during storage at 31° F. in 3 seasons between 1930 and 1935*

Lot No.	Delay at 50° F. before storing	Break-down in—						
		1930–31			1931–32	1934–35		
		Slight	Severe	Total	Total	Slight	Severe	Total
	Weeks	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	[2] 0	0.0	0.0	0.0	0.0	7.2	21.7	29.0
2	1	0	0	0	3.3	5.6	63.0	68.6
3	2	0	0	0	22.1	16.2	48.5	64.7
4	3	2.5	0	2.5	51.0	7.4	77.8	85.3
5	4	7.3	15.3	22.6	67.7	6.6	71.5	78.2
6	5	12.4	32.8	45.0	75.5	4.2	90.9	95.2
7	6	10.4	13.9	24.4	79.6	7.4	84.6	92.0
8	7	13.5	24.5	38.1	61.3	10.9	86.2	97.0
9	8	9.0	37.7	46.7	57.3	6.4	83.4	89.7
10	9				31.5	14.4	72.5	86.9
11	10				44.6	13.1	80.9	94.0
12	11					46.7	33.3	80.0
13	12					35.6	44.2	79.8

[1] Source of fruit: 1930, Apple Grove orchards, central Iowa; 1931, Highland fruit farm, western Iowa; 1934, Schurk orchards, southeastern Iowa. Growing conditions: 1930 and 1931, temperature high, soil moisture low; 1934, temperature very high, orchard heavily irrigated. Picking dates: Oct. 6, 1930; Oct. 7, 1931; Sept. 18, 1934. Fruit examined: Mar. 30, 1930; Feb. 25, 1931; Feb. 11, 1934. Number of boxes: 1931 and 1934, 1; 1932, 2.

[2] Stored same day picked in 1930 and 1931; 6 days' delay in 1934.

The difference between Grimes Golden and Golden Delicious in their response to long-delayed treatment must be considered in relation to varietal characteristics, i. e., date of maturity and length of storage season. Grimes Golden, for example, matures relatively early and begins its storage period early, whereas Golden Delicious matures late, at a

time when the average growing temperatures are lower. The conditions to which the two varieties are subject during the maturation period are decidedly different, and the fact that their ripening periods are not coincident may alone be sufficient to account for different responses in storage. Experiments similar to the foregoing were carried out with Golden Delicious in three other seasons, but no soggy break-down developed, indicating again that seasonable differences in the susceptibility of apples must be considered in studying this disease.

Results with Northwestern Greening

Results with Northwestern Greening for the season 1929–30 are given in table 8. The fruit, which was obtained from central Iowa, was selected and handled as in former seasons. The data indicate a marked lowering in the susceptibility of the apples when storage was delayed 2 weeks at 50° F. With longer delay practically no break-down developed at either 31° or 36° F. The results are similar to those for Jonathan, which consistently exhibits resistance with delayed treatments longer than 3 and 4 weeks.

RELATION OF MATURITY OF FRUIT ON DATE OF PICKING TO SUSCEPTIBILITY TO SOGGY BREAK-DOWN

Earlier results published by the writers (*13*) indicated that the maturity of Jonathan on the date of picking influences its susceptibility to soggy break-down. An interpretation of the earlier results, together with those for three other seasons, is summarized in table 9. The pickings recorded for each year extended throughout the commercial picking season for Jonathan, so that intermediate dates represent fairly closely the optimum picking dates. The term "highest maturity" indicates the degree of maturity attained on the latest picking date; the terms "intermediate" and "lowest maturity" refer to maturity attained on intermediate and earliest picking dates. All samples were stored at 32° F.

TABLE 8.—*Effect of length of period of delay before storage upon the percentage of soggy break-down* [1] *occurring in Northwestern Greening apples during storage at 31° and 36° F., 1929–30*

Lot No.	Delay at 50° F. before storing	Apples [2]	Break-down at storage temperature of—		Lot No.	Delay at 50° F. before storing	Apples [2]	Break-down at storage temperature of—	
			31° F.	36° F				31° F.	36° F.
	Weeks	*Number*	*Percent*	*Percent*		*Weeks*	*Number*	*Percent*	*Percent*
1	(3) 0	100	62.0	18.7	6	5	96	0.0	0.0
2	1	72	23.6	1.4	7	6	64	0	0
3	2	138	3.7	0	8	7	100	0	0
4	3	96	1.0	0	9	8	88	0	0
5	4	96	3.1	0					

[1] Fruit examined January 23.
[2] Number also indicates size of fruit.
[3] Stored same day picked, October 8.

In Jonathan, the highest degree of maturity as indicated by late picking appears not to be correlated with greatest susceptibility; and fruit picked at the beginning of the commercial season, or before it had become overmature, appears to be more susceptible to soggy break-down. These results agree in part with those reported by Tiller and

Chittenden (*20*) of New Zealand, who found that overmature Jonathan apples were less susceptible to "deep scald" than fruit of lesser degrees of maturity.

TABLE 9.—*Relation of degree of maturity of the fruit to soggy break-down susceptibility in Jonathan apples stored at 32° F.*

Season	Pickings per season	Degree of maturity associated with lowest susceptibility	Degree of maturity associated with highest susceptibility
	Number		
1919–20	5	Highest	Lowest.
1920–21	6	Lowest	Intermediate.
1922–23	5	Highest	Lowest.
1923–24	4	Lowest	Intermediate.
1924–25	3	Highest	Do.
1926–27	4	Intermediate and highest	Lowest.

The results of varying the picking maturity of Jonathan throughout three seasons are given in table 10. In 1923, the highest resistance was found in the earliest samples harvested on September 18; the lowest resistance in fruit of intermediate maturity harvested on September 24; and progressive increases in resistance were noted in the two succeeding pickings of fruit.

The 1924 results were similar in that highest maturity resulted in low susceptibility. Because of the slight occurrence of soggy break-down the data for 1926 give less information, but they do indicate greatest susceptibility in one of the most immature samples.

The value of these results with Jonathan lies mainly in that they show that picking maturity seems to affect susceptibility to soggy break-down, and that storage behavior may be directly related to the maturity attained on the tree.

TABLE 10.—*Percentage of soggy break-down occurring in apples of several varieties harvested at various stages of maturity and stored at 32° F.*

Variety	Year	Picking date	Break-down after indicated delay before storing of—			
			None	1 week	2 weeks	3 weeks
			Percent	*Percent*	*Percent*	*Percent*
Jonathan	1923	Sept. 18	0.0	4.5	----------	----------
		Sept. 24	45.5	82.2	----------	----------
		Sept. 29	34.6	14.0	----------	----------
		Oct. 4	13.5	12.3	----------	----------
	1924	Sept. 30	10.2	8.6	3.0	0.0
		Oct. 7	18.3	3.4	.3	0
		Oct. 14	4.8	2.9	1.7	0
	1926	Sept 20	0	3.4	0	0
		Oct. 2	0	0	0	0
		Oct. 5	0	0	0	0
		Oct. 12	0	0	0	0
Wealthy	1923	Aug. 27	0	8.9	12.0	----------
		Sept. 4	0	4.0	1.0	----------
		Sept. 11	0	2.0	0	----------
	1924	Aug. 28	0	0	.6	----------
		Sept. 3	0	.6	4.3	----------
		Sept. 9	0	0	10.4	----------
		Sept. 17	0	22.8	31.8	----------
Grimes Golden	1928	Sept. 7	0	----------	----------	44.9
		Sept. 11	0	----------	----------	65.3
		Sept. 14	0	----------	----------	61.4
		Sept. 20	0	----------	----------	89.0
		Sept. 25	.4	----------	----------	67.9
Northwestern Greening	1923	Sept. 12	0	4.4	4.7	----------
		Sept. 19	8.0	3.6	----------	----------
		Sept. 25	19.3	28.8	2.0	----------
		Oct. 17	51.2	----------	----------	----------

With Wealthy, the results for the two seasons are conflicting. In 1923 the most immature fruit was the most susceptible, and in 1924 the most mature fruit was the most susceptible.

With Grimes Golden, the highest susceptibility was found in fruit picked on September 20. As judged by the usual methods for determining maturity, this date was considered the optimum picking time of the season. It should also be noted that very immature fruits of Grimes Golden, picked as early as September 7, were highly susceptible

With Northwestern Greening, undermaturity was associated with a much higher degree of resistance than overmaturity.

EFFECT OF CONTINUOUS MOVEMENT OF STORAGE-ROOM ATMOSPHERE AND OF STORING IN OPEN CONTAINERS ON SOGGY BREAK-DOWN

In 1924 the writers reported studies (*13*) in which recirculation of the storage-room atmosphere gave good control of soggy break-down in one season and only partial control in another. The apples used were Extra Fancy Jonathan, wrapped and packed in standard boxes. Other researches (*1*, *8*) pertaining to the application of ventilation as a control measure in Jonathan, showed that circulating the storage-room atmosphere and using ventilated containers did not give consistent results. The results of further studies on the effect of aerating storage rooms and the use of open containers are given in table 11. Each lot of fruit consisted of a quantity equal to a standard apple box. The fruit was of Extra Fancy grade and was medium in size. The type of open basket used is shown in figure 6.

With one exception all of the eight boxes under air movement developed more soggy break-down than the boxes in the unaerated room. A comparison of the efficiency of the open-wire-basket method of storing under moving-air conditions with the conventional apple-box method in a room with still air showed in six of eight instances more break-down in the wire-basket containers. When open and standard containers both under aeration were compared, conflicting results were obtained.

TABLE 11.—*Effect of aerating storage rooms upon the percentage of soggy break-down occurring in Jonathan and Grimes Golden apples stored at 30° and 32° F. in two types of containers, season 1923–24*

Variety and storage temperature (°F.)	Picking date	Delay before storing	Break-down in storage room, air artificially circulated		Break-down in storage room, air not circulated, apples wrapped; in standard boxes
			Apples not wrapped; in open-mesh wire baskets	Apples wrapped; in standard boxes	
		Days	*Percent*	*Percent*	*Percent*
Jonathan, 32	Sept. 18	2	0.0	6.4	0.0
		8	52.6	69.8	63.2
	Sept. 24	2	14.0	14.9	4.1
		8	58.7	48.5	35.0
	Sept. 29	4	24.0	31.3	8.4
		7	9.6	2.3	3.0
	Oct. 4	4	23.7	16.5	.9
		8	35.3	26.0	18.8
Grimes Golden:					
30	Sept. 25	14	16.1	67.8	----
		21	13.6	60.3	----
32	do	14	12.2	73.5	----
		21	2.5	67.9	----

Strikingly different results were obtained with Grimes Golden at 30° and 32° F. In all instances the wrapped fruits in standard boxes developed more soggy break-down than those in open-mesh baskets. It should be noted, however, that the Grimes Golden in the open

FIGURE 6.—Method of storing Jonathan apples in wire baskets in aeration studies. Fruit in these baskets, under continuous atmospheric movement and at the same temperature, became more severely affected with soggy break-down than wrapped fruit in standard boxes in rooms without atmospheric movement. Dissimilar results were obtained with Grimes Golden.

baskets were somewhat shriveled at the end of the experiment, although not sufficiently so to affect their market value. The Jonathan fruits were not noticeably shriveled. The results tend to confirm those reported by the writers in an earlier experiment (*15*), in which storing Grimes Golden in open-wire baskets gave complete control of soggy break-down.

The comparison of fruits in open and closed containers with and without circulating storage-room atmosphere is not comprehensive enough for final conclusions. It may reasonably be expected, however, that the methods of storing and aerating used will not control soggy break-down in Jonathan. The results with Grimes Golden give more promise, although complete control was not obtained.

OTHER ETIOLOGIC FACTORS

Throughout the course of the investigations reported above, certain other etiologic factors of soggy break-down have received consideration. These include the effect of (1) fruit color, (2) nitrate fertilizers, (3) climatic conditions, and (4) size of fruit. Studies of all of these factors are not complete and final conclusions cannot be given.

TABLE 12.—*Effect of degree of fruit color upon the percentage of soggy break-down occurring in Jonathan and Northwestern Greening apples* [1] *during storage at different temperatures*

Variety	Picking date	Storage temperature	Color designation [2]	Stored immediately		Storage deferred 2 weeks	
				Apples	Break-down	Apples	Break-down
		° F.		*Number*	*Percent*	*Number*	*Percent*
Jonathan	Oct. 2, 1928	30	High	134	8.2	138	21.7
			Low	138	0	150	10.7
		32	High	110	1.8	113	16.8
			Low	138	0	149	8.1
		34	High	150	0	138	5.1
			Low	113	0	110	3.6
Northwestern Greening	Sept. 24, 1925	30	Yellow	217	54.3	.....	.....
			Green	288	16.0	.....	.....
		32	Yellow	151	84.1	.....	.....
			Green	255	30.9	.....	.....
		34	Yellow	173	74.5	.....	.....
			Green	250	36.4	.....	.....

[1] Date of examination, March 1 each year. Apples in oiled wraps in single boxes.
[2] The term "high color" for Jonathan indicates fruit completely colored or with 100-percent color; "low color," fruit 25 to 50 percent colored. In the case of Northwestern Greening, the yellow fruit was a deep golden yellow, the green about an apple-leaf green. Both yellow and green fruit were selected from the same trees on the same day.

Some effects of the degree of color of the fruit on susceptibility are given for Jonathan and Northwestern Greening in table 12. The results for one season suggest that color may influence susceptibility to some extent. Jonathan with 100-percent color in storage at 30° F. was less resistant than fruit with only 25- to 50-percent color. In Northwestern Greening at all three temperatures, fruit of full yellow color was much more susceptible than leaf-green fruit. However, the green fruit was not entirely resistant. In other experiments with Grimes Golden and Golden Delicious the writers have noted that green fruit was often more resistant than well-colored fruit, but not invariably so.

The results of 3 years' work on the influence of nitrogenous fertilizers on soggy break-down have been published (*12*), and while further studies have been made, the results merely confirm those already reported. In the earlier work (*12*) it was found that in wet years nitrate fertilizer seemed to increase susceptibility considerably in both Grimes Golden and Jonathan, but not in the 2 subsequent dry years (1930 and 1931). Similar experiments during 1932 and 1933

in four different orchards, with Grimes Golden, Jonathan, and Golden Delicious, have shown no further consistent increase in susceptibility of these varieties when the trees were fertilized with nitrate of soda. However, the continuous abnormal growing conditions from 1930 to 1933 resulting from high temperatures and drought, should be considered in interpreting the results, for under such conditions apples become more resistant. Drought conditions made it necessary to discontinue the study in 1934. In view of these facts, the writers do not consider the question of the relationship of nitrogenous fertilizers to the liability of apples to soggy break-down adequately answered.

In most of the varieties that are susceptible to soggy break-down it has been observed that the size of the fruit is of little consequence. In the majority of the tests the apples were sorted according to size before they were packed, so that the size factor has been definitely under observation throughout. The results to date indicate that small apples are fully as liable to soggy break-down as large ones. The one exception thus far noted is Northwestern Greening. The results of experiments with two size classes of this variety are recorded in table 13. The size class 64 to 104 proved to be much more susceptible than class 113 to 150. The differences noted between large and small fruit can probably be explained on the basis of maturity, since large Northwestern Greenings are generally considered to be more advanced in maturity on the tree than small Greenings. The range in size in this variety averages wider than most varieties. Large Northwestern Greenings, then, appear to be more susceptible to soggy break-down than small.

TABLE 13.—*Effect of fruit size upon the percentage of soggy break-down occurring in Northwestern Greening apples*[1] *during storage at different temperatures, season 1928–29*

Lot No.	Delay at 50° F. before storing	Break-down in large fruit (sizes 64 to 104)[2]				Break-down in small fruit (sizes 113 to 150)[2]			
		30° F.	32° F.	34° F.	36° F.	30° F.	32° F.	34° F.	36° F.
	Days	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	0	62.8	57.5	48.5	6.3	46.3	28.8	10.4	8.7
2	7	15.9	12.8	12.5	2.0	5.1	8.9	0	0
3	14	21.6	1.0	5.8	1.9	4.9	2.4	.8	0
4	21	3.8	.7	2.9	5.3	2.4	2.4	0	.8

[1] Date picked, Oct. 20; date of examination, Mar. 22.
[2] Various treatments resulted in no break-down in common storage.

THE PERIOD OF DEVELOPMENT OF SOGGY BREAK-DOWN

The development period of soggy break-down in Grimes Golden was reported in an earlier publication (*15*) to extend approximately from December 12 to February 15. The disease developed rapidly between January 11 and February 15, but there was no subsequent development up to May 12.

The method of storing Jonathans in open wire baskets, described above, afforded an opportunity to note the periodic development of soggy break-down in this variety. The fruit in the open wire baskets used in the aeration experiment (table 11) was carefully inspected at weekly intervals in the storage room to determine the initial appearance and the severity of the disease in all lots under treatment. The

data obtained (table 14) show that the dates of first appearance of the disease in the different baskets ranged from November 20 to January 3. It is evident, therefore, that soggy break-down may appear as early as 2 months after storage. Early appearance of the disease and high susceptibility seem to be associated.

TABLE 14.—*Date of initial appearance and maximum percentage of soggy break-down attained in Jonathan apples in open wire containers when stored immediately and after 1 week's delay, at 32° F., season 1923–24*

Lot number	Picking date	Break-down under immediate storage		Break-down when storage was delayed 1 week	
		Initial appearance	Maximum	Initial appearance	Maximum
			Percent		*Percent*
1	Sept. 18	None	0	Nov. 20	52.6
2	Sept. 24	Dec. 3	14.0	...do....	58.7
3	Sept. 29	...do....	24.0	Jan. 3	9.6
4	Oct. 4	Dec. 18	23.7	Dec. 3	35.3

Other studies with Jonathan have indicated that the period of development terminates toward the end of the commercial storage period. For example, published data (*13*) show that very little break-down developed between February 17 and April 1 in 1920, or between January 28 and March 28 in 1921. Other observations (table 15) show no development after January 26 in 1924, and still others show practically none after February 1. The period of development of the disease in Jonathan terminates rather abruptly, as it does in Grimes Golden. Although less is known of the period of development in other varieties, there are indications that it is similar to that of Grimes and Jonathan.

Further evidence that soggy break-down has a definite period of evolution in Jonathan was obtained by observing the increase in area of diseased tissue in marked specimens. On November 15, when break-down was first noted, 100 Jonathan apples were removed from storage, the affected portions were outlined with a sharp-pointed, moistened indelible pencil and the fruit was then returned to storage. The method of outlining the diseased areas and the progressive development from November 15 to December 3 are shown on two typical specimens in figure 7. In no case did the diseased areas expand after January 1.

TABLE 15.—*Extent of soggy break-down in Jonathan apples, stored at 32° F. after different periods of delay; examined on Jan. 26 and Mar. 12, 1924*

Lot No.	Picking date	Apples	Delay before storing	Break-down on—		Lot No.	Picking date	Apples	Delay before storing	Break-down on—	
				Jan. 26	Mar. 12					Jan. 26	Mar. 12
		Number	*Days*	*Percent*	*Percent*			*Number*	*Days*	*Percent*	*Percent*
1	Sept. 30	688	1	10.2	0	7	Oct. 7	676	14	.3	0
2		652	7	8.6	0	8		675	21	0	0
3		663	14	3.0	0	9	Oct. 11	688	1	4.8	0
4		651	21	0	0	10		625	7	2.9	0
5	Oct. 7	639	1	18.3	0	11		651	14	1.7	0
6		651	7	3.4	0	12		676	21	0	0

On January 14 of the same season 500 diseased Jonathan apples were marked and immediately returned to cold storage. An examination on February 15 revealed no further increase in the marked areas. It is recognized that the conditions to which the marked apples were subjected were not the same as those of other fruit kept continuously in storage containers. However, the results indicate that the advance of soggy break-down may be easily checked and that this disease has a rather definite period of development. In the latter respect it differs from apple scald, Jonathan spot, and mealy break-down, which continue to develop before and after the fruit is removed from storage. This information is of interest to commercial

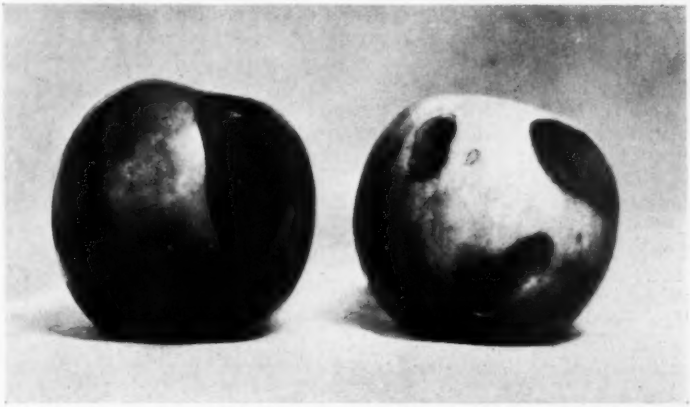

FIGURE 7.—Jonathan apples marked to show development of soggy break-down. Affected areas did not increase in size from December 3 to April 3. Apples were put back into cold storage immediately after marking. Typical external appearance of soggy break-down of Jonathan. (See fig. 1 for internal appearance of soggy break-down.)

storage operators and growers who may naturally suppose that most storage diseases of apples increase progressively in severity as the storage period is prolonged.

DISCUSSION

The observations recorded on the effects of picking maturity and delayed storage suggest that soggy break-down in apples may be associated with the stage of metabolism attained by the fruit at the time it is stored. Further evidence of this is brought out in the respiration studies of Harding (*4*, *5*) on large samples of Grimes Golden, and the storage studies carried out concurrently on similar fruit by Plagge (*11*). Harding found that respiratory activity in Grimes Golden accelerated rapidly at 50° F. during the first few weeks after picking. If actively respiring fruits of Grimes Golden were stored at 30°F., they subsequently became highly susceptible to soggy break-down; but if the fruits were stored before a high respiratory level was attained, or after it had subsided, they were resistant to the disease. When the fruit was stored at 36°F., regardless of its respiratory activity, it was resistant.

Other evidence that the stage of metabolic activity is important is shown by the results of many storage experiments with Jonathan and

Grimes Golden. In earlier experiments (*13*) with Jonathan, the writers observed that apples which had become severely affected with Jonathan spot were only slightly susceptible to soggy break-down. More recent studies confirm these observations. Jonathan fruits delayed 2 to 3 weeks before storing develop much spot and little or no soggy break-down, while those stored more promptly develop little Jonathan spot but more break-down. Jonathan apples susceptible to spot have already attained a high metabolic rate when stored, since this disease is closely allied to senility. Fruit that is susceptible to soggy break-down, therefore, is probably not so far advanced in its respiratory cycle when stored as that which is susceptible to Jonathan spot.

Similarly, a study of the tendency of Grimes Golden to apple scald and soggy break-down yields evidence that the stage of metabolism on the date of storage is indicative of its susceptibility to soggy break-down. In studies (*4*, *11*) in which respiratory activity in Grimes Golden was shown to be closely correlated with break-down susceptibility, break-down fruit was not affected with apple scald, or vice versa. This was observed a number of times with Grimes Golden and other varieties, and recently Brooks and Harley (*2*) have called attention to a similar result with Grimes Golden.

The results with Winter Banana further indicate that delayed storage may give different results in different seasons, and that no rule can be formulated as to the extent of delayed treatment that will result in maximum susceptibility.

The results of the storage-temperature studies over a period of years emphasize the need for raising slightly the apple storage temperatures used in commerce. Experiments year after year have shown that susceptible varieties store satisfactorily at 36° F., and that similar fruit may be severely affected with soggy break-down when the temperature is lower. Some may prefer to emphasize prompt storage rather than change the temperature, but such a practice is not always possible, and it will not serve as a satisfactory remedy in all cases, or even consistently in a few. The alternative is to store apples at 35° to 36° F. instead of at the conventional 31° to 32° range. Other reasons for making this change in the storage temperature were presented in an earlier publication (*17*).

Results of aeration studies with Jonathan, in which the storage-room atmosphere was kept continuously in motion around wrapped fruit in closed boxes, as well as around unwrapped fruit in mesh-wire baskets, offer little hope that circulating the storage-room atmosphere would be of value in the control of soggy break-down. Although some beneficial results from aeration methods have been recorded, it is believed that these may have followed from the more rapid rates of cooling in open containers and aerated storage rooms, with the net result of a changed metabolism, or from the resulting more even temperature control rather than from the removal of products of respiration. Whitehouse (*21*) pointed out that 160 hours (6½ days) was required to cool the center of a box of wrapped apples to 34° from 70° when the room temperature was 32° F., and that unwrapped fruit in boxes cooled more rapidly than wrapped. Moreover, a difference in time of only 3 days in the rate of cooling may markedly change the break-down susceptibility, especially when apples are in a high metabolic state.

The removal of certain harmful products of respiration deserves some consideration, particularly in the light of recent studies by Kidd and West (7), who point out that a toxic substance (probably ethylene) from ripe applies accelerates respiratory activity. But in the experiments described above Jonathan fruits sometimes developed more break-down in open containers than in closed boxes, and more in closed boxes under moving air than in still air. In these experiments, recirculating the storage-room air would probably not remove from the apples small traces of certain gases such as ethylene.

SUMMARY

Experiments carried on over a period of years on the effects of various causal factors of soggy break-down in apples, including picking maturity, storage temperature, short and long prestorage delays, and aeration are reported.

The maturity of the fruit on the picking date was shown to affect its tendency to break-down, but not always in the same direction for different varieties.

Storing promptly after picking frequently caused Jonathan, and usually Northwestern Greening, to be more susceptible to soggy break-down, while similar treatment of Grimes Golden, Wealthy, and Golden Delicious usually caused these varieties to be more resistant. Three seasons' work with Winter Banana showed a response similar to that of Jonathan.

With protracted delay before storing, i. e., from approximately 5 to 10 weeks at 50° F., Jonathan, Grimes Golden, Winter Banana, and Northwestern Greening exhibited marked resistance to soggy break-down. With similar treatment Golden Delicious developed more soggy break-down than the varieties just named, though it, too, tended to be resistant.

Results with picking-maturity and prestorage-delay experiments suggest that susceptibility to soggy break-down is associated with the stage of respiratory activity attained by the fruit at the time it is placed in storage.

Continuous movement of the storage-room atmosphere either over packed boxes of wrapped fruit or over fully exposed unwrapped fruit was unsatisfactory as a control measure for soggy break-down.

Studies on the periodic development of soggy break-down indicated initial and final dates of occurrence and a rather definite period of development.

Jonathan with 100-percent color in storage at 30° F. was more susceptible to soggy break-down than fruit of similar maturity having 25- to 50-percent color. Northwestern Greening of a full yellow color was more susceptible than distinctly green-colored fruit.

Of the varieties studied, Northwestern Greening was the only one in which large fruits were found to be more susceptible to soggy break-down than small fruits.

Apple varieties from the same orchards in different years, and from different orchards in the same year, exhibit marked differences in susceptibility to soggy break-down.

Investigations carried on for a number of years indicate that the most satisfactory method of controlling soggy break-down in all susceptible varieties under all the conditions tested, is to store apples at 36° F. rather than at lower temperatures.

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SOME PROPERTIES OF POTATO RUGOSE MOSAIC AND ITS COMPONENTS [1]

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INTRODUCTION

Rugose mosaic was described by Schultz and Folsom (*20*, *21*,[2]) as one of several mosaic diseases of the potato (*Solanum tuberosum* L.) at a time when the idea that one host might have more than one virus disease of the mosaic type was somewhat new. Since then plants affected with the rugose mosaic disease have been shown to contain at least two viruses, one of which may be called the pure rugose mosaic

FIGURE 1.—Connecticut Broadleaf tobacco plant showing, in leaf *a*, faint vein clearing resulting from infection by pure rugose mosaic.

virus (fig. 1). The other, which has been designated the latent virus, is present in practically all American potatoes, as can be shown by inoculating plants of certain other species of Solanaceae. (See figs. 2 to 4 and also *3, p. 8; 9, fig. 5, B; 11, figs. 2, A and 3; 22, figs. 4 and 44; 17, fig. 9*). Vein banding, first described by E. M. Johnson (*8, pp. 299–300 and pl. X, fig. 1*) on Burley tobacco (*Nicotiana tabacum* L.), and later given as a symptom of rugose mosaic on Turkish tobacco

[1] Received for publication April 22, 1937; issued December 1937.
[2] Reference is made by number (italic) to Literature Cited, p. 782.

(*23, pls. 2, 3*), is not a symptom of pure rugose mosaic on Connecticut Broadleaf tobacco or Green Mountain potato. By some recent writers the pure rugose mosaic component of rugose mosaic is referred to as the "vein-banding virosis," sometimes being designated as "vein-banding virus" and sometimes as "vein banding." Rugose mosaic is said to be common and destructive in commercial potatoes in some regions (*13, p. 5*), and has been found by the writers attacking many new seedling varieties in breeding plots.

Property studies were begun by the writers with the twofold purpose of increasing the information on viroses, and developing simple physi-

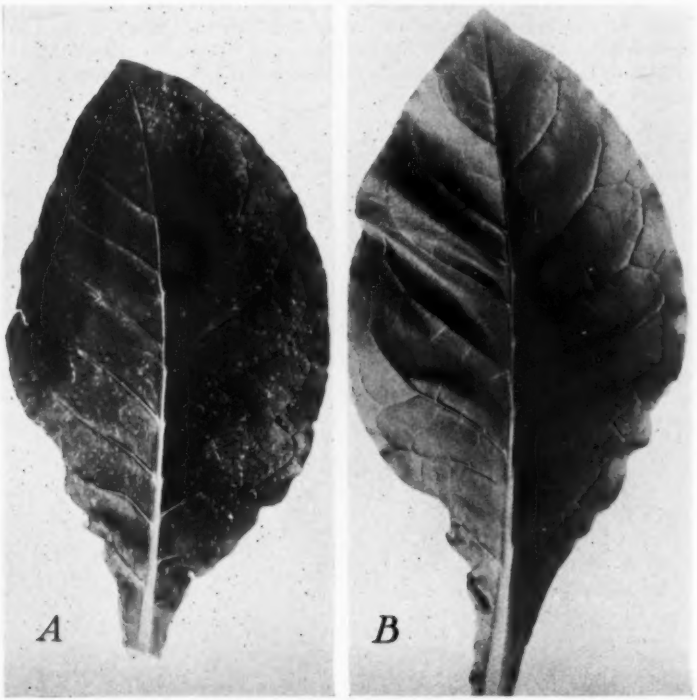

FIGURE 2.—*A*, Connecticut Broadleaf tobacco leaf from plant inoculated when young with juice from apparently healthy Green Mountain potato plants; *B*, uninoculated, healthy check. Photographed March 23, 1920.

cal and chemical tests for diagnosing potato viroses in local or imported lots of seed tubers. Several thousand plants in some hundreds of series were inoculated.

Although these property studies are concerned primarily with rugose mosaic and its components, comparisons were made between rugose mosaic and several other more or less similar virus diseases, such as tobacco spot necrosis, potato streak, and potato mild mosaic.

In potato plants of the Green Mountain variety in the normal Maine summer, the rugose mosaic disease is characterized by somewhat diffuse mottling, distinct dwarfing, rugosity premature death of

the plant, considerable reduction in yield of tubers, ease of infection by artificial methods, and slowness of natural translocation of the virus through infected plants and tubers.

A sequence of symptoms of rugose mosaic, typical of certain conditions, should be mentioned. Leaf-mutilation inoculation of young potato plants often produces, as the first symptoms in Green Mountain potatoes, in Maine, more necrosis than mottling. Streaking of veins and petioles and collapse and dropping of leaves occur at some distance above the inoculated leaves. The lower leaves, that is, the leaves below those that are subject to streaking and other forms of necrosis, show no symptoms, though their extract produces infection; the upper or youngest leaves merely develop mottling (*9, fig. 1, D*; *11, fig. 1, B*; *20, pl. 4, C*). Smith (*22, pp. 311–314, and fig. 50*), describes similar effects produced by virus Y. Apparently the rugose mosaic virus, upon becoming generally distributed through the plant, affects the leaves differently according to their age. Occasionally similar effects result from the delayed diffusion of the virus from the seed pieces of a tuber unit into growing plants (*21, pl. 8, C*), and hills and branches often display such effects as first symptoms.

FIGURE 3.—Connecticut Broadleaf tobacco leaf from plant inoculated when young with juice from apparently healthy Green Mountain potato plants. For different effects of the same inoculation, on a nother plant, see figure 24, and for healthy check see fig. 2, *B*. Photographed March 23, 1920.

A similar sequence of symptoms may occur in Connecticut Broadleaf tobacco. Vein clearing and necrosis (fig. 5) occur, as in the potato, as initial symptoms, in leaves above those inoculated. They sometimes occur in the lower or proximal end of one leaf, in the middle of the next higher leaf, and in the upper or distal end of the following leaf.

(See also the following: *9, fig. 6, B; 11, fig. 4, B; 22, fig. 3; 23, pl. 3.*) Inasmuch as the first part of a tobacco leaf to become full-formed is the distal, and the last part the proximal, leaf tissues which display the initial vein-clearing symptom are probably in approximately the same stage of development. Older tissues show no symptoms and younger tissues are merely mottled, or even apparently healthy, especially as the plant grows older.

FIGURE 4.—Connecticut Broadleaf tobacco leaf of plant infected with latent mosaic from apparently healthy Green Mountain potatoes. Note the increasing severity of the symptoms from the distal and oldest end of the leaf, to the proximal end.

Such a sequence in Connecticut Broadleaf tobacco infected with pure rugose mosaic (free of the latent mosaic), is less conspicuous because there is less vein clearing and no necrosis (fig. 1).

It is not known whether the difference in severity of symptoms in leaves and parts of leaves of different ages in these tests was due to a difference in the amount of virus that developed or to a difference in reaction to similar amounts of virus. Acquired immunity could hardly be the explanation, though it might account for the absence of severe symptoms upon reinoculation of rugose mosaic potato plants that are diseased through virus perpetuation by the tubers. Price (*19*) has presented evidence of acquired immunity in tobacco propagated vegetatively after infection by a tobacco ring spot virus which was then found to be present in smaller amounts than in the leaves that had been inoculated artificially and showed the severest symptoms. However, in the case of rugose mosaic it is not the inoculated leaves

that show the severest symptoms, but rather some of those infected through systemic spread of the virus.

Potato extract often had an immediate and direct toxic effect upon tobacco leaves. This effect was more pronounced when rugose mosaic

FIGURE 5.—Connecticut Broadleaf tobacco plant showing vein clearing and rugosity in three younger leaves and collapse in one leaf, resulting from infection by rugose mosaic, a composite of pure rugose mosaic and latent mosaic.

was present in the potato plant that supplied the inoculum, but it could not be used as an indicator of rugose mosaic infection. It consisted of puckering, chlorosis or bleaching, and collapse and drying

of the interveinal tissue, all limited to the vicinity of the place of inoculation. It was prevented by filtration through a Berkefeld N candle even when the filtered extract caused rugose mosaic infection.

SOURCE OF INOCULUM AND CHOICE OF HOSTS

The usual source of inoculum was stock of the Green Mountain potato that had been perpetuated in tuber-unit disease-maintenance seed plots from the time that it was selected as typical of rugose mosaic potatoes. Tobacco plants, after infection by artificial inoculation, were used to some extent as a source of inoculum.

Inoculated plants were usually of the Green Mountain variety of potato, the Connecticut Broadleaf variety of tobacco, or jimsonweed (*Datura stramonium* L.). Tobacco was more susceptible to rugose mosaic than potato. Jimsonweed was not infected by the pure rugose mosaic component, but was ideal for testing properties of the latent mosaic component (*17, figs. 4–6*).

Less satisfactory preliminary results were obtained with plants of the Bonny Best variety of tomato (*Lycopersicon esculentum* Mill.), nightshade or wonderberry (*Solanum nigrum* L.), husk-tomato or groundcherry (*Physalis pubescens* L.), and apple-of-Peru (*Nicandra physalodes* (L.) Pers.).

Tomato, when infected with rugose mosaic as proved by inoculation back to potato, showed symptoms similar to those produced by the latent mosaic. Nightshade was mottled by the latent mosaic and, when rugose mosaic was present, showed more conspicuous mottling in addition to wrinkling and leaf dropping. Husk-tomato, when infected with rugose mosaic, was more wrinkled and rugose than when infected with the latent mosaic alone. Apple-of-Peru was dwarfed and blotched by the latent mosaic, and in addition was dwarfed, mottled, chlorotic, and wrinkled when infected with rugose mosaic. Plants of bean (*Phaseolus vulgaris* L.) were immune to both rugose and latent mosaic, or at least did not become infected when inoculated by the leaf-mutilation method.

METHODS OF INOCULATION

The leaf-mutilation method was most satisfactory for inoculating potato plants. The young leaves of plants about 10 cm high were rolled between the palms of the hands until they were considerably bruised, and they were then painted with the inoculum, in the form of expressed juice, by means of a cotton swab held in flamed tweezers. Sometimes the juice was applied with the fingers and sometimes it was poured onto the bruised leaves.

For inoculating tobacco and jimsonweed, the simplest method was satisfactory and consisted of using two new wooden pot stakes from those to be used in marking the plants of the series. One stake was used to support the leaf and the other to paint the inoculum upon the leaf surfaces (dorsal and ventral) and to bruise the leaf somewhat. Normally the three largest leaves were inoculated when the plant had six leaves a centimeter or more in length.

If inoculum was scarce, instead of the stake-painting method a diseased leaf was used, either as picked or after having been pulped in a mortar. The inoculum was held in flamed tweezers and the

inoculated leaf was supported with a flamed knife. A slice of tuber, used in this way, was not so effective as leaf tissue.

Other methods tried and found less satisfactory were painting with a soft brush, pricking with a needle, and cutting, pruning, or stabbing with a knife, each in addition to applying the inoculum. After a finger-painting method had been used, washing the fingers with soap did not make them noninfectious to jimsonweed, although it did to tobacco and husk-tomato. This confirms the conclusion of Fernow (*5*) as to the unreliability of soap and water as a virus disinfectant. Inoculation of stems and roots was less effective than inoculation of leaves.

In the greenhouse, where some potato plants and all plants of other species were inoculated, the temperature usually was held at 18° to 24° C. by thermostatic control.

When potato plants were inoculated, there was usually a control series that contained plants representing the respective tuber units to which the inoculated plants belonged. However, this proved to be an unnecessary precaution. Sometimes the tubers of inoculated plants were saved and planted and the second generation of plants observed, but when young plants were inoculated with rugose mosaic this was found to be unnecessary.

The control series developed very little uncontrolled or chance transmission, either in greenhouse or field.

INFECTIVITY OF THE VIRUS

INOCULUM FROM DIFFERENT ORGANS

Confirmation was attempted of a previous result obtained in testing different parts of the potato plant, namely, that the infectiousness of the rugose mosaic virus seemed to be correlated with the amount of chlorophyll present (*21, p. 507*). In one new experiment, extract from leaflets or from petioles and stems was completely effective on potato, while extract from colorless sprouts or seed tubers infected only one-sixth of the plants inoculated. In a second experiment, extract from the foliage was completely effective on potato, extract from colorless sprouts or seed tubers infected five-twelfths of the inoculated plants, and extract from roots infected only one-sixth. When seed tubers were greened by exposure to light, the amount of greening was not correlated with variations in infectiousness of the extract. However, clarifying extract from foliage by appropriate filtration sometimes reduced infectiousness.

McKinney (*15, p. 35*) found the concentration of virus in mosaic tobacco to be much greater in leaf extract than in stem extract. In potatoes affected by spot necrosis, which is about the same as rugose mosaic, Valleau and E. M. Johnson (*23, p. 484*) found colorless sprouts noninfectious while the green leaves were highly infectious. Matz (*16*) reports lower rates of infectiousness by sugarcane mosaic from extract of white tissues than green tissues of the same shoots.

AGE OF PLANT SUPPLYING THE INOCULUM

Inoculum was secured July 21 to 31, about midway between planting and harvesting, from rugose mosaic potato plants grown in the field. Such inoculum was more infective than inoculum secured previously or subsequently. Further, inoculum from young plants infected all

inoculated plants, and symptoms appeared in 14 to 20 days. With inoculum from leaves midway along the stem of old plants 4 feet long and dying, 80 percent of the inoculated plants were infected and symptoms first appeared in 31 days. Inoculum from dry leaves of a plant dying 2 days previously from old age produced no infection.

In several comparisons, the age of tobacco plants with rugose mosaic had very slight if any effect upon the infectiousness of their extract when it was diluted with enough distilled water to reduce the proportion of extract to 10, 1, and 0.1 percent of the inoculum.

Diseased tobacco leaves dried for 8 months were not infectious.

AGING OF INOCULUM IN VITRO

Usually extract, to be used as inoculum, was obtained by grinding the plants through a food chopper, collecting the pulp in gauze, and squeezing out the sap by hand into a glass or porcelain container. The extract was then stirred well and divided into equal portions, and these were allowed to stand, or "aged," in vitro until used for inoculation. The important results of several illustrative comparisons are given in table 1.

Apparently aging in vitro often increased the infectiousness of rugose mosaic inoculum up to 4, 6, or 8 hours of aging, depending upon the series, while further aging decreased infectivity until it was lost. This loss in infectiousness required more aging at 5° C. than at 17° to 30°. At 30° the loss developed earlier with potato than with tobacco as the inoculated plant, occurring respectively before 5 days and after 10 days. At about 23° for potato, infectiveness was lost in 3 days by one inoculum, in 4 days by another, and in 5 days by others, while being retained by one for at least 7 days. At 15° for tobacco, infectiveness was lost in 7 days.

Increase in infectiousness of extract after a certain amount of aging in vitro has been reported for tobacco mosaic by Elmer (*4*), Olitsky and Forsbeck (*18*), and McKinney (*14, p. 8*), and for curly top by Bennett (*1, table 15, second, ninth, and last sources*).

Mosaic virus of the latent type sometimes resisted aging slightly better than that of rugose mosaic, and aging was not an effective means of freeing pure rugose mosaic from latent.

TABLE 1.—*Effect of aging, at normal and at low temperatures (° C.), upon the infectivity of rugose and latent mosaic extracts from potato and tobacco*

Series [1]	Virus	Source of inoculum	Plants inoculated	Aging of inoculum	Results
1	Rugose mosaic	Potato	Potato	None 4 hours 8 hours 24 hours 30 hours	All hills infected, most completely and severely with 4 hours' aging. Symptoms appearing later with 24–30 hours' aging.
2	do	do	do	None 4 hours [2] 8 hours [2] 24 hours [2] 30 hours [2]	All hills infected, most completely and severely with 8 hours' aging. Delay of appearance of symptoms, with 24–30 hours' aging, greater than in series 1.
3	do	do	do	None 8 hours 24 hours 2 days 7 days	All hills infected. Increased infection. Decreased infection progressively.

[1] Series 1–5 and 8 were in the field, and the rest in the greenhouse, at about 23° C.
[2] In sunlight for at least 8 hours, for comparison with series 1, kept in the dark.

TABLE 1.—*Effect of aging, at normal and at low temperatures (° C.), upon the infectivity of rugose and latent mosaic extracts from potato and tobacco*—Contd.

Series	Virus	Source of inoculum	Plants inoculated	Aging of inoculum	Results
4	Rugoes mosaic	Potato	Potato	None	No difference; all hills infected.
				2 days	
				3 days	Decreased infection.
				4 days	No infection.
				5 days	
				8.5 days	
				11 days	
5	do	do	do	None	All hills infected.
				1 hour	Most infection at 4 and 6 hours.
				2 hours	
				4 hours	
				6 hours	
				8 hours	
				3 days	Infection with aging at 5° but not at 17°, 23°, and 20°.
				5 days	
				7 days	No infection with aging at any temperature.
				8 days	
				11 days	
				17 days	
				38 days	
6	do	do	do	5 days	Infection with aging at 5° but not at 30°.
				13 days	No infection.
7	do	do	Tobacco	5 days	Infection decreased with aging at 30° as compared with 5°.
				7 days	
				10 days	
8	do	do	Potato	None	Infection increased up to 4–6 hours, decreased in 8 hours, lost at 5 days.
				2 hours	
				4 hours	
				6 hours	
				8 hours	
				5 days	
9	do	do	do	17 days	Infection with aging at −6°.
				5 days	No infection with aging at 20°.
				7 days	
				10 days	
10	do	do	Tobacco	8 days	88 percent infected with aging at −18° in frozen shoot.
				17 days	No infection with aging at 5° but all infected with aging at −7°.
				20 days	33 percent infected with aging at −7° in frozen shoot.
				36 days	No infection with aging at −7° in frozen shoot.
11	Latent mosaic	do	Jimsonweed	17 days	All infected with aging at −7° in frozen shoot.
[3] 12	Rugose mosaic	do	Tobacco	None	All infected, most with symptoms in 13 days.
				1 hour	
				2 hours	
				3 hours	
				4 hours	
				5 hours	
				6 hours	
				7 hours	All infected, minority with symptoms in 13 days.
				8 hours	All infected, none with symptoms until after 13 days.
				1 day	
				2 days	
				3 days	Majority infected.
				4 days	
				5 days	
				6 days	Minority infected.
				7 days	None infected.
				8 days	
				9 days	
				10 days	

[3] Aging at about 15° C.

Aging in potato extract in vitro usually inactivated rugose mosaic inoculum within a few days, according to James Johnson (*9, table II*) and James Johnson and Grant (*10, table 7*), both authorities finding the latent type persisting somewhat longer. Similar results are reported

by Koch (*11, table 8*).[3] Van der Meer (*17, table 4*) reported persistence of the latent mosaic for less than 6 days in one test, and for nearly 3 weeks in others.

TEMPERATURE

A common method of determining the so-called thermal death point of a virus is to immerse a tubeful of inoculum for a certain length of time in water maintained at a constant temperature. This requires frequent readjustment of the water bath, or the use of several baths, if a comparison is to be made of the effects of different temperatures on parts of the same inoculum. The writers heated a water bath gradually, removing and cooling the sublots of inoculum as the rising temperature, registered by a thermometer in the inoculum, reached various points. Many such comparisons were made but need not be given here. The results of several typical comparisons are shown in table 2.

TABLE 2.—*Effect of high temperatures on infectivity of rugose mosaic inoculum from potato*

Series	Plants inoculated	Temperature regulation, ° C.		Results
[1] 1	Potato	Raised about 10° every 8 minutes to.	50	Complete infection.
			60	Little infection.
			70	No infection.
			80	Do.
2	Tobacco	Raised about 10° every 4 minutes to.	50	Complete infection.
			55	Do.
			60	Do.
			65	No infection.
			70	Do.
			75	Do.
			80	Do.
			85	Do.
			90	Do.
3	Potato	Raised to	45	Complete infection.
			50	Do.
			55	Do.
			60	No infection.
			65	Do.
			70	Do.
4	Tobacco	Raised to	60	All latent mosaic.
			65	Do.
			80	80 percent latent mosaic.
			95	No infection.
5	do	Raised to	40	Complete infection.
			45	Do.
			50	Do.
			55	83 percent infected.
			60	Only latent mosaic.
			65	Do.
			70	Do.

[1] Series 1 was in the field; the rest were in the greenhouse.

Rugose mosaic virus usually was inactivated by raising the temperature to 60° or 65° C., or by holding the temperature for 10 to 15 minutes at 55°. Even with conditions apparently the same from series to series, the results were not alike. A higher temperature was required for tobacco extract used on tobacco than for transfers from potato to potato, potato to tobacco, or tobacco to potato. Exposures to temperatures just below those able to inactivate increased the time required for the first symptoms to appear and decreased the percentage

[3] He refers to vein banding, ring spot, and mottle; in a later publication he and Johnson state that "For all practical purposes, potato 'rugose mosaic' is due to the vein-banding virus" (*12, p. 42*), though actually including mottle or ring spot in addition (*p. 41*).

of plants infected; no change in the nature of the symptoms was observed. The results with pure rugose mosaic were much the same as with rugose. The latent mosaic present in rugose mosaic had a higher limit of tolerance, sometimes persisting to 90°.

The thermal death point of rugose mosaic virus has been found by others to lie between 60° and 65° (*9, p. 11*), 55° and 60° (*10, table 3*), and 58° and 60° C. (*11, table 7*), all at 10-minute exposures to constant temperature. The corresponding limits of the latent mosaic were about 70° (*9, p. 11; 10, table 3*), 65° to 70° (*11, table 7*), and 70° to 75° (*17, table 5*).

DILUTION

Extract from diseased plants was diluted to various degrees with water of different kinds and with juice from healthy plants, with a resulting reduction in infectiousness, at least when a certain point of dilution had been reached. The results of the tests with diluted extracts, from different sources transferred to one host or another, are given in part in table 3. These results are representative of a large mass of data which show in general that infectiousness was lost when the proportion of extract in the inoculum had been reduced to between 10 and 1 percent by distilled water (in transmission tests from potato to potato in the greenhouse); to between 1.0 and 0.5 percent by healthy potato juice (potato to potato in the field); and to between 1.0 and 0.1 percent by soft lake water and distilled water (potato to tobacco in the greenhouse). Some infectiousness persisted at 1.0 to 0.1 percent in series diluted with hard river and well water (potato to potato in the field) and with distilled water (potato to tobacco and tobacco to tobacco in the greenhouse). As the limit was approached, there was a decrease in the percentage of plants infected by inoculation and an increase in the time required to bring out the first symptoms.

The latent mosaic was somewhat more persistent at the dilution inactivation point of rugose mosaic.

As compared with the above inactivation point of about 0.1 percent, or about one one-thousandth, it is interesting to note that the inactivation points reported by others for rugose mosaic are about one one-thousandths (*9, p. 11*) and one five-thousandths to one ten-thousandths (*11, table 9*), and for the latent mosaic, one ten-thousandths (*9, p. 11*), one ten-thousandths to one one-millionths (*11, table 9*), and about one one-hundred-thousandths (*17, table 6*).

The greater inactivating effect of healthy potato juice, as compared with water in series 2 and 3 of table 3, upon rugose mosaic, is similar to that reported by McKinney (*14, table 5*) for tobacco and cucumber extracts.

The inactivation of tobacco mosaic virus by pokeweed (*Phytolacca decandra* L.) juice in vitro (*14, p. 9*) was found to hold also for rugose but not for latent mosaic, and pokeweed juice painted onto tobacco leaves several days before inoculation with rugose mosaic reduced infection below that obtained on water-painted checks.

TABLE 3.—*Effect of dilution upon infectivity of rugose mosaic virus from potato*

Series [1]	Plants inoculated	Diluent	Percent extract in dilution	Results
1	Potato	River water	100	All infected.
			50	Infection of all, but progressively less severe.
			20	
			10	
			5	88 percent infected.
			2	20 percent infected.
			1	10 percent infected.
2	do	Well water	50	All infected.
			20	
			10	
			5	All infected; majority incompletely.
			2	90 percent infected.
			1	70 percent infected.
			.5	44 percent infected.
			.2	20 percent infected.
			.1	22 percent infected.
3	do	Healthy potato extract	50	90 percent infected.
			20	40 percent infected.
			10	30 percent infected.
			5	20 percent infected.
			2	33 percent infected.
			1	11 percent infected.
			.5	None infected.
			.2	
4	do	Distilled water	50	All infected.
			10	
			1	None infected.
			.1	
			.01	
5	Tobacco	do	50	All infected.
			10	
			1	83 percent infected.
			.1	None infected.
			.01	
6	do	do	5	60 percent infected.
			1	None infected.
			.5	40 percent infected.
			.1	None infected.
			.05	
			.01	
			.005	
			.001	

[1] Series 1–3 were in the field; the rest were in the greenhouse. Series 2 and 3 were kept under similar conditions.

FILTRATION

Rugose mosaic potato extract filtered through Berkefeld N and V candles infected a small percentage of inoculated tobacco plants, but usually no potato plants. The percentage of infection was not increased by previously clarifying the extract by filtration through paper that had become coated with the gelatinous mass of crushed plant tissue, or filter slime. Neither was it increased by filtration under 80 to 90 pounds' pressure per square inch in a Hill pressure apparatus. Here the unfiltered sediment in the rubber bag enlosing the candle retained its full infectiousness, although exposed to the pressure indicated. (More than 75,000 pounds' pressure was required to inactivate mosaic tobacco extract (7).) Clarification did not inactivate the extract, although sometimes it reduced infectiousness slightly. Of 10 potato plants inoculated in the field with extract filtered under air pressure through a Chamberland B filter, 1 became diseased, possibly through uncontrolled natural infection. Of 18 potato plants inoculated in the greenhouse with clarified extract filtered through a Berkefeld V candle with suction that reduced the pressure to about one-fifth of an atmosphere, 1 became diseased. Filtration through a Berkefeld V candle had little effect on the infectiousness of latent mosaic extract.

CHEMICALS

Each chemical to be tested was dissolved in water in a series of different concentrations in equal amounts of solvent. Then the solution at each concentration was combined with an equal amount of fresh extract. For example, to get a 5-percent concentration, a 10-percent solution of the chemical was made up and added to an equal amount of the fresh extract. Thus the resulting mixture contained a 50-percent concentration of the extract and half the concentration of chemical in the original solution. The mixing was followed by inoculation within about 10 minutes. The order of mixing and inoculation was according to decreasing concentration of the chemical. The chemicals tested are representative of several important types of compounds, and are readily available, easily prepared for use, and for the most part, of recognized value as disinfectants or fungicides. The results are given in table 4.

The lethal point of hydrochloric acid for rugose mosaic virus varied with conditions. It was between 0.07 and 0.2 percent in series 4 (in transmission tests from potato to potato in the field); between 0.2 and 0.5 in series 11 (potato to potato in the greenhouse), in series 12 and 13 (potato to tobacco in the greenhouse), and in series 14 (tobacco to potato in the greenhouse); and beyond 0.5 percent in series 15 (tobacco to tobacco in the greenhouse). However, the closeness of results probably was more remarkable, considering the different conditions, than the variation. The lethal point of sodium hydrate also varied. In the order of decreasing concentration needed to inactivate, the tested chemicals and their inactivation points were as follows: Ethyl alcohol (C_2H_5OH), over 50 percent; sodium chloride (NaCl), about 5 percent or 1 to 20; formaldehyde (HCHO), about 0.5 percent or 1 to 200; hydrochloric acid (HCl) and sodium hydrate (NaOH), respectively, about 0.2 percent or 1 to 500; and copper sulphate ($CuSO_4$) and sulphuric acid cleaning fluid, respectively, about 0.1 percent or 1 to 1,000. Prolonging the duration of exposure to the chemical to an hour did not shift the point much (series 17 versus 18).

TABLE 4.—*Effects of chemicals upon infectivity of rugose mosaic virus from potato and tobacco*

Series [1]	Source of inoculum	Plants inoculated	Chemical	Percent of chemical	Results
1	Potato	Potato	C_2H_5OH	5 and less	All infected.
				10	Mostly infected.
				25 and 50	Some infected.
2	do	do	C_2H_5OH [2]	As in series 1	As in series 1.
3	do	do	HCHO [3]	2 and more	No infection.
4	do	do	HCl [4]	0.04	All infected.
				0.07	20 percent infected.
				0.2 and more	No infection.
5	do	do	NaOH	0.1	50 percent infected.
				0.2 and more	No infection.
6	do	do	NaCl	0.1 to 1	All infected.
				2	50 percent infected.
				5	10 percent infected.
7	do	do	HCHO	0.004 to 0.2	80 to 100 percent infected.
				0.3	50 percent infected.
				0.7 and more	No infection.

[1] Series 1–8 were in the field; the rest were in the greenhouse.
[2] Commercial "denatured" material was used in series 2.
[3] Percentages calculated on basis of actual HCHO; thus percent in terms of the 40-percent solution used would be 5 and more.
[4] Percentages calculated on the basis of actual HCl; thus each is only about two-fifths of concentration of the c. p. material used, with specific gravity 1.19.

TABLE 4.—*Effects of chemicals upon infectivity of rugose mosaic virus from potato and tobacco*—Continued

Series	Source of inoculum	Plants inoculated	Chemical	Percent of chemical	Results
8	Potato	Potato	$CuSO_4$	0.1	80 percent infected.
				0.2	No infection.
				1 and more	Do.
9	do	Tobacco	C_2H_5OH	20 and less	All infected.
				50	13 percent infected.
10	do	do	HCHO	0.2	All infected.
				0.5	40 percent infected.
				1 and more	No infection.
11	do	Potato	HCl	0.1 to 0.2	40 percent infected.
				0.5	No infection.
12	do	Tobacco	HCl	0.1	All infected.
				0.2	20 percent infected.
				0.5	No infection.
13	do	do	HCl	0.1	33 percent infected.
				0.2	17 percent infected.
				0.5	No infection.
14	Tobacco	Potato	HCl	0.1 to 0.2	All infected.
				0.5	No infection.
15	do	Tobacco	HCl	0.1	60 percent infected.
				0.2	81 percent infected.
				0.5	33 percent infected.
16	Potato	do	NaOH	0.05 to 0.1	All infected.
				0.2	No infection.
17	do	do	NaOH	0.05	All infected.
				0.1	40 percent infected.
				0.2	60 percent infected.
[5] 18	do	do	NaOH	0.05 to 0.1	All infected.
				0.2	No infection.
19	Tobacco	do	do	0.05 to 0.1	All infected.
				0.2	80 percent infected.
20	Potato	do	NaCl	1	All infected.
				5	14 percent infected.
				10	No infection.
21	do	do	$CuSO_4$	0.1	17 percent infected.
				0.2	No infection.
				0.5 to 1	Plants killed.
22	do	do	Cleaning fluid [6]	0.05 to 0.1	No infection except by latent component.
				0.25 to 2.5	No infection.
[7] 23	do	Jimson weed	do	0.05 to 0.1	Latent mosaic.
				0.25 to 2.5	No infection.
24	do	Tobacco	do	0.01 to 0.1	All infected.
				1	No infection except by latent component.
25	do	do	NaOH	0.1	20 percent infected.
				0.2	All infected.
				0.5	None infected.
				1	

[5] Same inoculum as for series 17 except mixed 1 hour before being used.
[6] Made up with sulphuric acid and potassium bichromate.
[7] Series 22 and 23 were under the same conditions.

The lethal point for the latent component did not differ much from that for rugose mosaic except for greater resistance of the latent to the toxic effects of formaldehyde and the cleaning fluid.

James Johnson (*9, p. 12*) and Koch (*11, table 10*) report similar results with alcohol and HCHO. Van der Meer also found alcohol a weak inactivator (*17, table 7*). Freeman (*6*) found that from rugose mosaic only the latent mosaic component could be recovered at pH 4 to 5.5 and at 9.7; from pH 5.6 to 7.6 there was no inactivation, and at pH 3.6 or lower not even the latent component could be recovered.

COMPARISON OF RUGOSE MOSAIC WITH OTHER MOSAICS

MILD, CRINKLE, AND LEAF-ROLLING POTATO MOSAICS

Leaf-mutilation inoculation of bean plants with potato mild mosaic and leaf-rolling mosaic extract produced no symptoms. Stake-painting inoculation of potato plants with potato mild mosaic extract did

not always infect and, when it did, produced late and therefore unsatisfactory symptoms. Similarly, unsatisfactory symptoms resulted on potato with the potato crinkle and leaf-rolling mosaics.

TOBACCO MOSAIC

Dried leaves from the laboratory of B. M. Duggar in St. Louis, Mo., from experiments of F. M. Blodgett at Cornell University, Ithaca, N. Y., and from a field in Connecticut, were used as the sources of tobacco mosaic inoculum which gave infection on potato plants. The symptoms were not those of a mosaic, but were rather like those of streak with some suggestion of blackleg, and included necrotic spotting and streaking of the leaves, streaking of the stem, leaf dropping, wilting, and early death of the plant. These results agree with those reported by Fernow (*5, table 21 and pl. VII*) and Blodgett (*2*). The St. Louis strain, used on jimsonweed, caused a slight amount of spot and streak necrosis at first but soon became masked, though still present in virulent form as was shown by transfer back to tobacco.

STREAK

Although causing no mottling of Green Mountain potatoes, streak produced mosaic on tomato and Spaulding Rose potato plants, and when on tobacco, nightshade, and apple-of-Peru, was not to be distinguished by symptoms from rugose mosaic. Jimsonweed, immune to rugose mosaic, also was immune to streak, and the raising of the temperature to 60° C. inactivated streak in vitro. The inactivating effects of pokeweed juice on rugose mosaic were duplicated in tests repeated with streak. These inoculation studies suggest that the streak virus is very similar to the virus of rugose mosaic. A tomato plant inoculated on February 3, 1927, and used for successful return inoculation to potato, was still vigorous on October 17, 1928 (fig. 6).

TOBACCO SPOT NECROSIS

Tobacco leaves infected with spot necrosis were kindly supplied the writers by James Johnson, of the Wisconsin Agricultural Experiment Station, and in Maine they produced a disease on greenhouse potato and tobacco very similar to rugose mosaic but somewhat more virulent and infectious. A second supply from Johnson consisted of both tobacco and potato spot necrosis leaves which were used on greenhouse tobacco in comparison with inoculum from Maine rugose mosaic potato. Here the tobacco-leaf inoculum was more infective and had a shorter incubation period than the other two inocula. However, when the three newly infected tobacco series were compared by inoculating potatoes in the field, the strain received from Johnson in the potato leaves was the only one that gave current-season symptoms, and it was less infectious than inoculum from field-grown rugose mosaic potato plants. Such effects of host upon infectivity are similar to those reported by Johnson (*9, table III*).

James Johnson stated (*9, p. 4*), in 1929, that after repeated attempts at correlation, he had concluded that his spot necrosis was identical with the rugose mosaic of Schultz and Folsom; that is, with the rugose mosaic used in the present comparisons. He also explained (*9, p. 23*) that "in previous literature from the Wisconsin laboratory

FIGURE 6.—Bonny Best tomato plant October 17, 1928, 20 months after infection with potato streak from Spaulding Rose potato plants and 18 months after back-inoculation to Green Mountain potato plants that resulted in the death of the plants from streak. This photograph was taken shortly after a second virus also a mosaic, had spread through the plant, and shortly before the plant succumbed.

referring to 'rugose mosaic' of Triumph potatoes, this name should read 'crinkle mosaic'."

ATTENUATION AND INCREASE IN VIRULENCE

The idea that a virus may increase in virulence is met many times in the study of mosaics, including those of potato. A tuber-propagated host would seem to be promising material from which to obtain evidence. In the writers' experience, an apparent increase in virulence such, for example, as mild mosaic apparently becoming rugose mosaic, has occurred chiefly in proximity to plants already showing the more severe disease, under conditions favorable to transmission, or, of course, in plants inoculated experimentally with the more virulent type of the virus. Isolation in effective insect cages has prevented an increase of virulence of mosaic, even in stocks perpetuated for about 20 years.

In greenhouse studies in Maine efforts to increase the virulence of latent mosaic until it resembled spot necrosis or rugose mosaic, by passage through as many as eight successive series of tobacco plants, have failed to bring about any change from one mosaic to the other. The symptoms of both kinds of mosaic vary. Leaf contact is sometimes sufficient to transmit either mosaic. The general tendency in virology is toward longer lists of viroses for various hosts and longer lists of means of transmission of the respective viroses, so that the chance of uncontrolled contamination by a severer type, through an unsuspected agency, seems more reasonable than a spontaneous sudden increase in virulence. In a discussion of this problem, Johnson (*9, pp. 18–21*) states that "the mottle form from healthy potatoes cannot ordinarily be changed to spot necrosis at will," and points out the possibility of accidental transmission of spot necrosis as the cause of a sudden increase in virulence.

Apparent attenuation of a virus often may be reasonably explained on the basis of (1) the elimination of one or more components of a composite or multiple virosis, (2) a decrease in the severity of symptoms after the first have developed or (3) a decrease in the severity of symptoms with certain changes in environmental conditions or with increasing age of the host plant.

SUMMARY

Potato rugose mosaic is a composite mosaic which includes pure rugose mosaic, or the vein-banding mosaic, and latent potato mosaic When contracted by a partly grown potato or tobacco plant, rugose mosaic often affects leaves and leaf parts differently according to their age; some become necrotic; older ones show no symptoms and younger ones develop mottling. Rugose mosaic aggravated a toxic effect of potato extract upon tobacco; the effect was eliminated by Berkefeld-candle filtration.

Rugose and latent mosaic were studied better on potato, tobacco, and jimsonweed than on tomato and several other species of the same family; bean was immune.

The most satisfactory method for inoculating potato plants was the leaf-mutilation method, and the best for inoculating tobacco and jimsonweed was a stake-painting method.

Extract from green shoots was more infectious than that from colorless sprouts, seed tubers, and roots of rugose mosaic potato plants, and was sometimes made less infectious by clarification.

With rugose mosaic, the age of potato plants or their parts had more effect than the age of tobacco plants in determining the infectiveness of their extracts. Drying of leaves soon eliminated the infectivity.

Aging in vitro for several hours progressively increased the infectiousness of rugose mosaic extract; further aging progressively reduced it, and under certain conditions the inactivation-point was reached in a few days. Infectiveness was inhibited later at low temperatures (5° C.), and later for tobacco than for potato as the inoculated host. The latent mosaic virus sometimes resisted aging longer than the pure rugose mosaic virus.

Rugose mosaic extract usually was inactivated when the temperature was raised to 60° or 65° C., or when the temperature was held for 10 minutes at 55°, but the thermal death-point varied with the species involved in the transfer and also with the series even when conditions apparently were similar. Pure rugose mosaic acted like the composite virus, but the latent component had a higher limit of tolerance.

Rugose mosaic extract became inactivated at about 1 to 0.1 percent upon dilution with water. Healthy potato juice had a slightly greater inactivating effect than water. Latent mosaic was somewhat more persistent than rugose. Pokeweed juice inactivated rugose mosaic extract but not latent mosaic.

The virulence of rugose mosaic virus was reduced considerably but not eliminated by filtering the extract through bacteria-retaining candles; the latent mosaic virus was affected only a little by the process. Neither 80 pounds' pressure nor clarification reduced the infectiousness of rugose mosaic extract more than a slight amount.

Latent mosaic was more resistant to formaldehyde and a sulphuric-acid cleaning fluid than rugose, but was similar in its response to other chemicals. The lethal point of HCl for rugose mosaic varied with conditions, within the range of about 0.07 to over 0.5 percent. To inactivate, the strength of ethyl alcohol had to be greater than 50 percent; of NaCl, about 5 percent; of HCHO, about 0.5 percent; of HCl and NaOH, about 0.2 percent; and of $CuSO_4$ and the cleaning fluid, about 0.1 percent.

Preliminary comparisons between mosaics on potato and other plants showed that the methods used for these property studies of rugose mosaic are unsatisfactory for mild, crinkle, and leaf-rolling mosaics of potato. Tobacco mosaic can infect Green Mountain potato plants. Potato streak and tobacco spot necrosis both resemble rugose mosaic.

Latent mosaic was not increased in virulence by eight successive passages through tobacco plants.

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RESPONSE OF OAT VARIETIES TO DIFFERENT FERTILITY LEVELS[1]

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INTRODUCTION

A 3-year rotation of corn, oats, and winter wheat has been grown at four fertility levels at the Ohio Agricultural Experiment Station at Wooster. Data were collected for all three crops, and those for corn and winter wheat have been published.[3] The present paper is concerned with the oat crop in this rotation.

The literature has been reviewed in the papers mentioned above, and repetition seems unnecessary. Suffice it to say that differential response of varieties to fertility level in many of our common crops has proved significant, and that this interaction deserves careful study.

OUTLINE OF EXPERIMENT

The 3-year rotation of corn, oats, and winter wheat was laid out on three fields of Canfield silt loam of low fertility. The higher fertility levels were obtained by adding one, two, and four increments of fertilizing materials. Actual applications are given in table 1. The oats received no direct applications of fertilizers.

TABLE 1.—*Plot treatments at the 4 fertility levels used in 3-year rotation tests with corn, oats, and winter wheat*

Fertility level	Treatment used for crop indicated		
	Corn	Oats	Wheat
A	None	None	None.
B	4 tons manure; 100 pounds 0-16-0 broadcast and 100 pounds 4-12-4 in hill.	None	200 pounds 2-14-4 in fall and 50 pounds nitrate of soda in spring.
C	8 tons manure; 200 pounds 0-16-0 broadcast and 200 pounds 4-12-4 in hill.	None	400 pounds 2-14-4 in fall and 100 pounds nitrate of soda in spring.
D	16 tons manure; 400 pounds 0-16-0 broadcast and 400 pounds 4-12-4 in hill.	None	800 pounds 2-14-4 in fall and 200 pounds nitrate of soda in spring.

The four levels were set up on adjacent strips in each field and the varieties sown across them. Oats were planted in triplicate; the size of plot was 5⅓ by 35 feet (0.00427 acre). The order of varieties in each replication was the same in 1929, random in the other 3 years. In all cases each variety was sown in a continuous strip across the fertility levels; thus the order of plots was identical on each level.

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[2] The writers wish to acknowledge the help of G. H. Stringfield, who, with R. M. Salter, set up the experiment. He selected the varieties and collected the data for the early years, and has offered helpful suggestions in the preparation of the manuscript.

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PRESENTATION OF DATA

Seventeen varieties of oats were grown for four seasons, 1929 to 1932, inclusive. Table 2 gives the grain yield, straw yield, and bushel weight for each variety-level-season unit. In figure 1 the average performance of all 17 varieties in each season has been plotted.

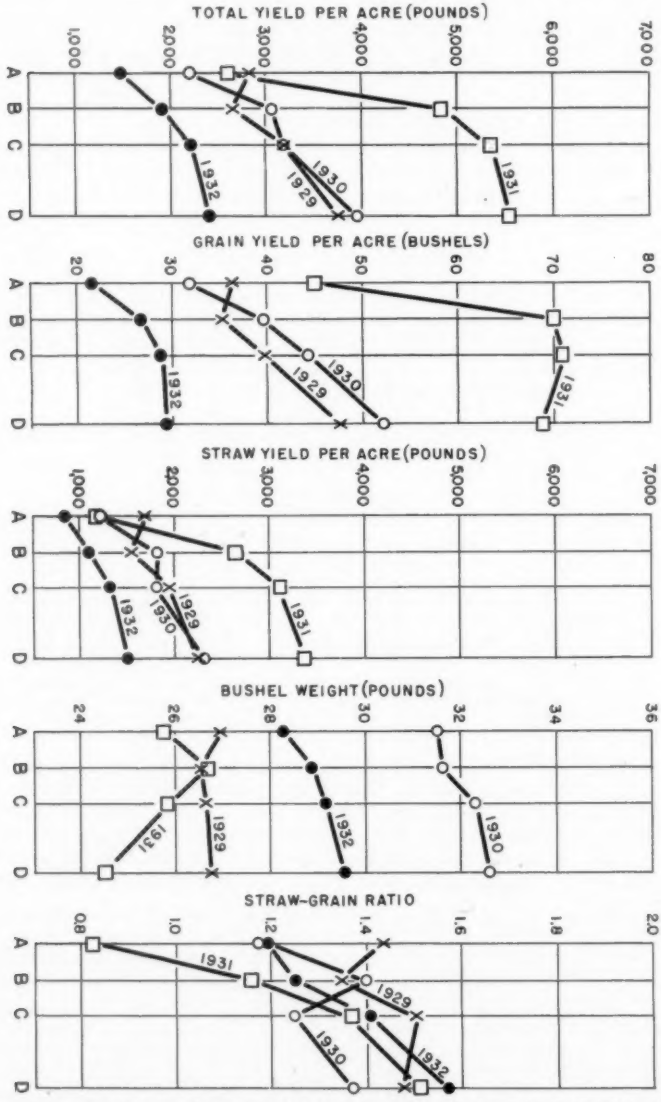

FIGURE 1.—Average response of 17 varieties of oats to four soil-fertility levels (A, B, C, D) during four seasons

TABLE 2.—*Yield per acre and weight per bushel of 17 varieties of oats grown at 4 levels of soil fertility, 1929–32*

Variety	Level	1929			1930			1931			1932			Average		
		Grain	Straw	Weight per bushel	Grain	Straw	Weight per bushel	Grain	Straw	Weight per bushel	Grain	Straw	Weight per bushel	Grain	Straw	Weight per bushel
		Bushels	*Pounds*	*Pounds*	*Bushels*	*Pounds*	*Pounds*	*Bushels*	*Pounds*	*Pounds*	*Bushels*	*Pounds*	*Pounds*	*Bushels*	*Pounds*	*Pounds*
Burt	A	32.9	1,444	26.2	26.6	852	30.4	42.2	1,220	25.0	21.5	860	28.9	30.8	1,094	27.6
	B	32.3	1,230	25.6	36.8	1,178	30.1	60.9	2,100	27.1	24.5	1,037	29.0	38.6	1,386	28.0
	C	36.0	1,815	26.0	42.7	1,365	31.8	71.1	3,240	26.7	27.8	1,399	28.5	44.4	1,955	28.3
	D	43.3	1,971	25.7	47.8	1,529	31.9	63.8	4,012	25.3	25.7	1,465	29.0	45.2	2,244	28.0
Albion	A	30.5	1,132	26.9	23.3	747	30.1	42.4	1,220	25.7	20.6	662	27.1	29.2	940	27.5
	B	29.9	1,073	26.9	27.7	887	31.6	69.7	2,360	26.5	26.8	855	28.8	38.5	1,294	28.5
	C	37.8	1,444	26.4	32.1	1,027	31.7	63.9	2,480	24.8	29.4	1,049	28.5	40.8	1,500	27.9
	D	43.9	1,717	26.9	40.5	1,295	32.1	61.8	1,980	24.8	29.4	1,161	28.6	43.9	1,538	28.1
Richland	A	33.5	1,269	27.4	22.6	755	30.4	40.4	720	25.8	20.8	759	27.3	29.3	876	27.7
	B	34.2	1,249	27.2	31.1	1,260	31.2	62.4	1,980	26.4	24.5	802	28.3	38.1	1,323	28.3
	C	37.2	1,464	27.0	34.3	1,470	31.1	63.0	2,500	24.8	25.7	913	28.3	40.1	1,587	27.8
	D	42.7	1,756	27.0	45.2	1,742	32.2	69.1	3,260	23.5	28.8	1,232	29.1	46.5	1,998	28.0
Fulghum	A	28.7	1,425	28.2	25.0	1,066	31.0	40.9	960	27.8	17.2	703	29.7	28.0	1,039	29.2
	B	32.9	1,366	28.0	32.8	1,517	31.6	63.1	2,200	29.1	22.0	953	30.6	37.7	1,509	29.8
	C	33.5	1,659	27.4	36.9	1,385	31.4	54.2	1,680	27.8	24.8	1,018	29.8	37.4	1,436	29.1
	D	39.6	1,776	27.4	46.4	2,248	31.8	77.5	1,900	25.7	25.7	1,301	29.7	47.3	1,806	28.7
Franklin	A	32.9	1,600	28.5	33.3	1,035	32.7	45.2	1,200	26.2	19.0	926	29.1	32.6	1,190	29.1
	B	40.9	1,815	29.1	39.6	1,844	32.3	72.5	2,660	28.2	25.4	1,116	30.5	44.6	1,859	30.0
	C	42.1	2,166	28.8	43.3	1,649	33.8	73.8	3,320	26.7	26.2	1,230	29.8	46.4	2,091	29.8
	D	46.4	2,186	28.4	53.0	2,271	33.1	79.1	3,700	25.9	25.1	1,548	29.5	50.9	2,426	29.2
Gopher	A	37.8	1,522	27.6	28.9	1,175	33.3	53.5	1,000	26.2	17.3	661	30.0	34.4	1,090	29.3
	B	38.4	1,347	27.7	36.0	1,727	33.1	78.1	2,740	25.8	23.1	838	30.6	43.9	1,663	29.3
	C	43.3	1,737	27.2	38.9	1,478	34.1	68.2	2,880	23.9	24.4	994	30.5	43.7	1,772	28.9
	D	48.8	1,952	27.0	49.8	2,061	34.6	51.2	2,800	24.0	28.3	1,211	30.3	44.5	2,006	29.0
Big Four	A	36.6	2,030	27.6	32.1	1,073	32.2	41.6	1,160	26.3	21.4	841	28.5	32.9	1,276	28.7
	B	42.1	1,776	27.5	41.6	1,937	32.3	67.8	2,600	26.7	24.7	990	28.8	44.1	1,826	28.8
	C	45.1	1,991	27.2	45.2	1,898	33.2	68.9	3,260	26.1	25.9	1,271	30.0	46.3	2,105	29.1
	D	51.2	2,186	27.0	53.2	2,341	33.4	63.7	4,120	24.9	28.1	1,450	30.3	49.1	2,524	28.9
Wayne	A	39.0	1,717	26.9	30.6	980	33.0	46.1	1,640	27.1	16.0	734	27.6	32.9	1,268	28.7
	B	40.9	1,581	27.0	41.6	1,330	32.9	74.5	2,680	28.2	23.0	1,006	28.6	45.0	1,649	29.2
	C	43.9	1,952	26.9	44.8	1,435	33.5	80.8	3,340	28.0	25.9	1,208	28.3	48.9	1,984	29.2
	D	50.0	2,225	26.8	53.2	1,704	33.6	65.2	4,700	25.7	26.3	1,343	29.7	48.7	2,493	29.0
Markton	A	32.3	1,542	25.4	39.1	1,470	32.6	47.4	1,220	26.1	18.7	716	28.3	34.4	1,237	28.1
	B	27.4	1,308	24.2	44.0	2,014	32.8	73.8	2,160	27.6	24.7	906	29.3	42.5	1,597	28.5
	C	31.7	1,639	25.1	51.5	1,696	33.6	74.6	2,760	26.2	24.7	1,187	30.5	45.6	1,821	28.9
	D	37.2	1,932	24.1	63.0	2,730	33.6	76.8	3,460	24.7	26.6	1,297	31.8	50.9	2,355	28.6
Miami	A	45.1	1,835	29.2	33.8	1,175	31.7	46.5	1,160	26.0	19.7	800	28.0	36.3	1,243	28.7
	B	36.6	1,561	27.9	39.4	2,085	31.7	71.2	2,480	26.4	26.1	1,040	28.6	43.3	1,792	28.7
	C	45.7	2,205	27.9	40.6	1,346	32.8	70.2	2,740	26.2	26.6	1,413	29.1	45.8	1,926	29.0
	D	54.9	2,537	28.3	51.0	2,723	32.1	71.9	3,940	24.2	26.9	1,457	28.4	51.2	2,664	28.3

TABLE 2.—*Yield per acre and weight per bushel of 17 varieties of oats grown at 4 levels of soil fertility, 1929–32*—Continued

Variety	Level	1929			1930			1931			1932			Average		
		Grain	Straw	Weight per bushel	Grain	Straw	Weight per bushel	Grain	Straw	Weight per bushel	Grain	Straw	Weight per bushel	Grain	Straw	Weight per bushel
		Bushels	*Pounds*	*Pounds*	*Bushels*	*Pounds*	*Pounds*	*Bushels*	*Pounds*	*Pounds*	*Bushels*	*Pounds*	*Pounds*	*Bushels*	*Pounds*	*Pounds*
Cornellian	A	47.0	2,088	29.3	36.0	1,338	32.8	48.1	1,020	26.9	25.7	898	29.6	39.2	1,336	29.7
	B	39.6	1,854	28.7	43.8	2,100	32.8	72.0	2,620	27.7	27.8	1,052	30.0	45.8	1,907	29.8
	C	49.4	2,244	28.6	50.1	1,742	33.0	68.7	3,340	26.9	30.0	1,360	30.6	49.6	2,172	29.8
	D	57.9	2,596	28.5	56.9	2,691	34.1	68.3	2,580	25.1	30.0	1,608	30.0	53.3	2,369	29.4
Silvermine	A	36.0	1,659	26.3	28.2	1,354	32.0	42.8	1,440	26.2	19.6	781	29.1	31.7	1,309	28.4
	B	36.0	1,425	26.1	36.7	1,626	32.0	69.0	3,320	27.3	25.6	1,140	29.1	41.8	1,878	28.6
	C	38.4	1,659	26.7	42.3	2,069	33.3	66.7	3,180	25.1	27.9	1,310	30.0	43.8	2,055	28.8
	D	45.1	2,147	26.3	46.4	2,248	33.0	71.1	3,340	25.5	26.3	1,447	30.5	47.2	2,296	28.8
Victory	A	42.1	1,776	26.9	36.5	1,322	31.5	45.3	1,200	25.6	36.5	883	28.0	40.1	1,295	28.0
	B	38.4	1,815	25.0	47.6	1,898	31.7	68.6	2,480	26.4	47.6	1,318	27.9	50.6	1,878	27.8
	C	37.8	2,147	26.4	53.2	2,264	31.9	77.7	3,260	26.7	53.2	1,549	28.5	55.5	2,305	28.4
	D	54.9	2,459	27.3	55.4	2,349	33.1	73.1	3,440	25.1	55.4	1,761	28.3	59.7	2,502	28.5
Lincoln	A	33.5	1,581	22.4	30.4	1,206	28.2	42.4	1,080	24.4	19.0	799	25.7	31.3	1,167	25.2
	B	29.3	1,483	22.3	40.6	1,657	27.7	66.4	2,620	25.1	24.9	1,109	26.2	40.3	1,717	25.3
	C	33.5	1,737	22.5	41.1	1,952	28.9	65.1	3,060	24.7	26.7	1,239	27.1	41.6	1,997	25.8
	D	42.7	2,225	21.8	48.6	2,015	28.9	66.9	2,920	23.2	23.6	1,500	27.6	45.5	2,165	25.4
Wolverine	A	35.4	1,444	27.6	31.6	1,400	33.5	48.2	940	24.3	21.6	809	27.4	34.2	1,148	28.2
	B	31.7	1,327	27.5	43.5	1,952	33.4	71.9	2,760	24.4	24.1	1,011	27.9	42.8	1,763	28.3
	C	39.0	1,639	27.2	45.5	1,890	34.5	74.6	3,300	25.2	27.0	1,337	28.2	46.5	2,042	28.8
	D	46.4	2,030	27.9	57.4	2,520	35.2	71.7	3,020	22.8	26.0	1,438	28.8	50.4	2,252	28.7
Ohio 201	A	37.2	2,166	25.5	39.4	1,618	29.7	45.1	1,360	23.7	24.3	964	28.1	36.5	1,527	26.8
	B	36.0	1,893	25.4	49.3	2,777	29.7	72.7	3,360	25.3	28.7	1,423	28.9	46.7	2,363	27.3
	C	41.5	2,654	25.7	53.5	2,722	30.4	74.4	4,100	25.3	31.1	1,604	29.1	50.1	2,770	27.6
	D	51.8	3,181	26.0	58.1	3,275	31.2	75.6	3,980	22.5	32.5	1,838	30.8	54.5	3,069	27.6
Ohio 19607	A	39.0	2,108	25.8	40.1	1,750	29.8	45.4	1,420	24.6	22.9	979	27.0	36.9	1,564	26.8
	B	34.8	1,776	26.2	45.0	2,528	29.5	74.8	3,220	25.2	27.1	1,413	26.0	45.4	2,234	26.7
	C	40.3	2,303	25.7	53.2	2,652	30.5	84.7	4,300	25.7	27.9	1,767	28.5	51.5	2,756	27.6
	D	51.2	3,123	27.1	60.0	2,979	31.0	70.4	3,760	23.4	28.9	1,688	29.0	52.6	2,888	27.6
Average	A	36.4	1,667	26.9	31.6	1,195	31.5	44.9	1,174	25.8	21.3	810	28.2	33.6	1,212	28.1
	B	35.4	1,522	26.6	39.8	1,783	31.6	70.0	2,608	26.7	26.5	1,059	28.8	42.9	1,743	28.4
	C	39.8	1,909	26.6	44.1	1,767	32.3	70.6	3,102	25.9	28.5	1,285	29.1	45.8	2,016	28.5
	D	47.5	2,235	26.7	52.1	2,278	32.6	69.2	3,348	24.5	29.0	1,456	29.5	49.5	2,329	28.3

Plots were cut with an ordinary grain binder, and uniform stubble was left on all levels. For this reason straw yields are relatively too low on the A level, where there was definitely less vegetative growth. The errors introduced by the unharvested stubble at the higher levels are of little importance.

A study of figure 1 shows that there were marked differences between seasons. In 1931 the yields of both grain and straw were high; in 1932 they were decidedly low. The influence of season must, therefore, be considered in interpreting the experiment. Table 3 gives meteorological data at Wooster for all four seasons.

TABLE 3.—*Mean maximum and minimum temperatures and precipitation for Wooster, Ohio, during the growing season for oats, 1929–32*

Month	Mean maximum temperature				Mean minimum temperature				Precipitation			
	1929	1930	1931	1932	1929	1930	1931	1932	1929	1930	1931	1932
	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	*Inches*	*Inches*	*Inches*	*Inches*
April	63.0	64.0	61.6	60.0	40.4	38.0	35.2	33.0	5.58	2.23	4.10	2.55
May	70.6	74.3	70.2	72.2	44.8	48.0	44.6	45.4	4.84	1.59	4.45	1.93
June	79.0	84.1	81.2	82.6	53.0	53.9	54.3	55.2	4.10	2.86	3.49	3.44
July	83.9	90.4	89.3	84.8	59.3	57.4	61.9	59.2	6.79	1.71	2.97	3.14
August	80.3	86.0	83.7	85.6	52.4	55.1	59.9	56.0	1.26	2.64	4.68	2.01

In 1929 there was a wet, relatively warm spring, followed by a dry period during the latter part of May and early June. Summer temperatures were not excessive, and rainfall was plentiful after mid-June.

The season of 1930 was somewhat cooler in early spring, and warm in summer. Precipitation was lower in April and early May. Through the rest of May and in June and July the weather was very dry.

In 1931 conditions were more favorable than in other years. Distribution of rainfall was very satisfactory, and in spite of high temperatures in late June and July oats yielded well. Test weight was low, however.

The temperature in the spring of 1932 was not satisfactory, and precipitation was low in May and early June. Oats made poor vegetative growth and gave low yields of grain.

ANALYSIS OF VARIANCE

Analysis of variance by the method developed by Fisher [4] offered a satisfactory means of handling the data. To estimate odds for significance Snedecor's F [5] was used.

Table 4 presents data from the analyses of total yield (grain+straw) and of grain yield for each season. Variety, level, block, and the variety-level interaction were considered. Because of the smaller area of land in one of the fields, three varieties were sown in only two blocks in 1930. Calculations were made on the actual data, and again with estimated yields introduced for the 12 missing plots. The theoretical third replication was given the mean yield of the two actually grown. Such a procedure would be expected to give too low

[4] FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 4, 302 pp., illus. Edinburgh and London. 1932.

[5] SNEDECOR, G. W. CALCULATION AND INTERPRETATION OF VARIANCE AND COVARIANCE. 96 pp. Ames, Iowa. 1934. (Iowa State Col., Div. Indus. Sci. Monog. 1.)

an estimate of variance, but in this case it changed the F values very little, and the significance not at all. Any error introduced, therefore, had no practical importance, and the estimated plot yields were used because they permitted the analysis of data for all seasons together with greater facility.

TABLE 4.—*Analysis of variance, by seasons, of total yield and of grain yield for 17 varieties of oats grown at 4 fertility levels in 4 seasons*

TOTAL YIELD

Season	Variance due to—	Degrees of freedom	Sums of squares	Variance	F	1 percent point for F [1]	5 percent point for F [1]	
1929	Variety	16	642.54	40.159	15.25	2.20	1.75	
	Level	3	657.59	219.197	83.22	3.94	2.68	$\bar{x}$=13.26 pounds.
	Block	2	102.39	51.195	19.44	4.78	3.07	σ=1.62 pounds.
	Variety-level interaction	48	88.25	1.839	.70	1.80	1.50	C. V.=12.22 percent.
	Error	134	352.94	2.634				
	Total	203	1,843.71					
1930	Variety	16	915.18	57.199	14.22	2.20	1.75	
	Level	3	1,594.80	531.600	132.17	3.94	2.68	$\bar{x}$=13.54 pounds.
	Block	2	43.76	21.880	5.44	4.78	3.07	σ=2.01 pounds.
	Variety-level interaction	48	121.95	2.541	.63	1.80	1.50	C. V.=14.84 percent.
	Error	134	538.91	4.022				
	Total	203	3,214.60					
1931	Variety	16	822.74	51.421	5.29	2.20	1.75	
	Level	3	5,153.23	1,717.743	176.56	3.94	2.68	$\bar{x}$=19.66 pounds.
	Block	2	32.32	16.160	1.66	4.78	3.07	σ=3.12 pounds.
	Variety-level interaction.	48	579.69	12.077	1.24	1.80	1.50	C. V.=15.87 percent.
	Error	134	1,303.68	9.729				
	Total	203	7,891.66					
1932	Variety	16	134.92	8.433	11.91	2.20	1.75	
	Level	3	399.95	133.317	188.30	3.94	2.68	$\bar{x}$=8.42 pounds.
	Block	2	54.43	27.215	38.44	4.78	3.07	σ=.84 pounds.
	Variety-level interaction.	48	21.55	.449	.63	1.80	1.50	C. V.=9.98 percent.
	Error	134	94.90	.708				
	Total	203	705.75					

GRAIN YIELD

1929	Variety	16	3,861.01	241.313	11.91	2.20	1.75	
	Level	3	4,659.80	1,553.267	76.69	3.94	2.68	$\bar{x}$=39.55 bushels.
	Block	2	1,403.19	701.595	34.64	4.78	3.07	σ=4.50 bushels.
	Variety-level interaction.	48	834.37	17.383	.86	1.80	1.50	C. V.=11.38 percent.
	Error	134	2,714.14	20.255				
	Total	203	13,472.51					
1930	Variety	16	6,451.46	403.216	18.89	2.20	1.75	
	Level	3	11,225.41	3,741.803	175.30	3.94	2.68	$\bar{x}$=41.94 bushels.
	Block	2	325.05	162.525	7.61	4.78	3.07	σ=4.62 bushels.
	Variety-level interaction.	48	439.09	9.148	.43	1.80	1.50	C. V.=11.02 percent.
	Error	134	2,860.28	21.345				
	Total	203	21,301.29					
1931	Variety	16	2,466.25	154.141	2.71	2.20	1.75	
	Level	3	23,822.65	7,940.883	139.37	3.94	2.68	$\bar{x}$=63.48 bushels.
	Block	2	266.39	133.195	2.34	4.78	3.07	σ=7.55 bushels.
	Variety-level interaction.	48	3,870.02	80.625	1.42	1.80	1.50	C. V.=11.89 percent.
	Error	134	7,634.61	56.975				
	Total	203	38,059.92					
1932	Variety	16	647.08	40.443	9.92	2.20	1.75	
	Level	3	1,495.39	498.463	122.29	3.94	2.68	$\bar{x}$=24.91 bushels.
	Block	2	591.88	295.940	72.61	4.78	3.07	σ=2.02 bushels.
	Variety-level interaction.	48	263.94	5.499	1.35	1.80	1.50	C. V.=8.11 percent.
	Error	134	546.12	4.076				
	Total	203	3,544.41					

[1] Approximate values.

Total yield represents the total top growth, independent of how much of the material moves into the developing kernels. Grain yield, on the other hand, measures particularly the translocation of elaborated plant food into the developing embryo and endosperm. A general comparison of the two parts of table 4 indicates that after variance is removed for the factors considered, residual error is greater for total than for grain yields. This is measured by the coefficient of variability (C. V.), since the standard deviation (σ) has been estimated from the variance for error. This is surprising, since the filling of the grain follows the virtual completion of vegetative growth, and grain yields, therefore, would be expected to show greater variability because of the additional physiological processes involved.

A second unexpected reaction appears in comparing the 1931 results with those of other years. This was the season when oats gave their highest yields in both straw and grain, but the coefficients of variability for both total yields and grain yields were the largest obtained because of the large variance for error. Conversely, the yields and coefficients were both lowest in 1932. This implies that factors introducing variability other than variety, level, and the interaction of these two, become increasingly important as the season is more favorable, and the yields are higher.

Differences among varieties were highly significant in all cases, but the F values were decidedly lower in 1931. This does not necessarily indicate that the spread among varieties was greater with the more adverse seasonal conditions. The greater unaccounted-for error in 1931 is largely responsible for the lower significance in that year.

Fertility was a very important factor because of the wide range between the A level and the D level. The spread among the different treatments, however, varied with season as was to be expected. Leaching, precipitation, and temperature probably all played important roles, especially since the oats crop received no direct applications of fertilizer.

A comparison of the variance for variety with the varietal means, for grain and for total yields, may be made by considering the ratio $\frac{\sqrt{\text{Variance due to variety}}}{\text{Mean varietal yield}}$. In all four seasons this ratio is greater for total than for grain yields. In 1929 it is 1.22 times; in 1930, 1.17 times; in 1931, 1.87; and in 1932, 1.33 times as great.

For optimum development oats require a cool, moist season. The Ohio climate is by no means ideal for them. It may be that the relatively cool weather before heading is favorable enough that the inherent differences between varieties can find expression in the vegetative growth, whereas the hot weather following heading is so unfavorable that varietal response in the grain yields is inhibited in some degree.

The variety-level interaction is one of the most important points studied. In no single season did it prove mathematically significant, though in 1931 the F value approaches the 5-percent point closely, especially for the grain yields. There is a more nearly significant interaction for grain than for total yields in 3 of the 4 years, indicating a possible differential response in the reproductive phases greater than in purely vegetative growth. The evidence for this, however, is far from conclusive.

Definite indication of differences between blocks was found, especially on that field used in 1929 and 1932. In fact, in most cases the variety-block interaction was significant and, strangely enough, in all instances except for grain in 1931, this interaction was more significant than that of variety-level. This represents a serious error in the experiment, which, however, cannot be justifiably removed, since it represents an uncontrolled and largely indeterminate factor. It is probably much less important as a source of error in the analyses presented in table 5, where all seasons are taken together. Since three fields were used, variance for block could not be removed directly, and the total yield of the three replicates was used as the starting point for this anaysis.

TABLE 5.—*Analysis of variance for total yield and grain yield of 17 varieties of oats grown at 4 levels of soil fertility during 4 seasons*

TOTAL YIELD

Variance due to—	Degrees of freedom	Sums of squares	Variance	F	1-percent point for F [1]	5-percent point for F [1]	
Variety	16	5,611.10	350.694	29.00	2.12	1.70	
Level	3	16,263.13	5,421.043	448.28	3.91	2.66	
Season	3	38,933.07	12,977.690	1,073.16	3.91	2.66	$\bar{x}$=13.72 pounds.
Variety-level interaction	48	692.99	14.437	1.19	1.75	1.40	σ=1.16 pounds.
Variety-season interaction	48	1,935.05	40.314	3.33	1.75	1.40	C. V.=8.45 percent.
Level-season interaction	9	7,153.55	794.839	65.73	2.63	2.00	
Error	144	1,741.33	12.093				
Total	271	72,330.22					

GRAIN YIELD

Variety	16	21,967.39	1,372,962	14.64	2.12	1.70	
Level	3	81,874.57	27,291.523	291.07	3.91	2.66	
Season	3	464,226.48	154,742.160	1,650.35	3.91	2.66	$\bar{x}$=42.47 bushels.
Variety-level interaction	48	2,720.43	56.676	.60	1.75	1.40	σ=3.23 bushels.
Variety-season interaction	48	18,310.02	381.459	4.07	1.75	1.40	C. V.=7.60 percent.
Level-season interaction	9	41,735.19	4,637.243	49.46	2.63	2.00	
Error	144	13,501.81	93,763				
Total	271	644,335.89					

[1] Approximate values.

Without question varieties differed in yielding ability, and the variance due to levels was large, but neither of these factors exerted as much influence as season. The variety-level interaction was not mathematically significant in any case. However, when variety-block and level-block variance was removed from error, this interaction reached the level of significance in one season in the case of total yields, and in three seasons in the case of grain yields. Nevertheless when all seasons were considered together, no significant variety-level interaction was found. This was in marked contrast to the wheat crop in the rotation, where, in the corresponding analysis, odds exceeded 99:1 for total yields, and approached the 1-percent point for grain yields.

Consideration of the data from all analyses indicates that for oats the variety-level interaction is of questionable significance mathematically, and the actual yield differences involved are of no practical

significance. It therefore appears safe to apply variety-test results obtained at Wooster to rather divergent soil-fertility conditions, since the unaccounted-for errors are relatively low.

SUMMARY AND CONCLUSIONS

A 3-year rotation of corn, oats, and winter wheat has been grown at four fertility levels at the Ohio Agricultural Experiment Station. Oats alone are considered in this paper.

Data on grain yield, straw yield, and weight per bushel are given for 17 varieties of oats, all grown for four seasons.

Climatological data covering the period of the experiment are included.

Analysis of variance for total yield and for grain yield, by individual seasons, and all seasons together, indicates that differences due to variety, to level, and to season are all highly significant. The variety-season interaction is highly significant, but the variety-level interaction is probably not significant.

Unaccounted-for error, as measured by the coefficient of variability, is less for grain than for total yield. This is unexpected, and may be due to the wide divergence of climate from the ideal for the crop.

Because the variety-level interaction is of questionable significance, and at the same time the unaccounted-for errors are low, it appears safe to apply results of a variety oat experiment at Wooster, Ohio, to a rather wide range of soil conditions.

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